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Molecular Advances and Targeted Therapies for Pediatric Central Nervous System Tumors

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Abstract

Central nervous system tumors are extremely rare in the pediatric population and molecularly heterogeneous. Growing scientific research and clinical practice experience are improving medical therapies to increase survival outcomes and quality of life and reduce side effects. The 2019 Neurobiology of Disease in Children Symposium, held in conjunction with the 48th annual meeting of the Child Neurology Society, aimed to (1) describe molecular advances in tumor classification, (2) better understand the evolution of targeted therapies, and (3) more clearly formulate a treatment plan for patients. The article summarizes the presentations and includes an edited transcript of a panel discussion.

Keywords

pediatric brain tumors, neurogenetics, molecular pathogenesis, therapies, medulloblastoma, cognitive effects, ependymoma

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Session I: Clinical Aspects

Overview of Clinical Aspects

Nada Jabado, MD, PhD; McGill University, Montreal, QC, Canada.

Dr Nada Jabado presented an overview of clinical aspects of managing pediatric brain tumors. Brain tumors are the top cause of cancer-related deaths and the most common solid tumors in children. There are many subgroups of pediatric brain tumors, like medulloblastoma and ependymoma, that remain some of the deadliest forms of human cancer. Over the years, there have been improved outcomes for many tumor entities and more children are surviving into adulthood. However, despite improved therapeutic management and care, the short- and long-term consequences of survivorship are still severely debilitating. Radiation therapy has multiple side effects, but most importantly, it impacts endocrine function, development, and learning processes. These lifelong side effects often prevent survivors of pediatric brain tumors from full social integration and living a fulfilled life as adults.

There are a few challenges in diagnosing pediatric brain tumors. Pediatric brain tumors are a diagnostic challenge for neuropathologists as many tumors are histologically similar but molecularly distinct. Additionally, pediatric brain tumors are completely molecularly distinct from adult brain tumors. These tumors are directly linked with brain development. They have exquisite spatio-temporal distribution and hijack regional growth factors to enable tumor growth. They also have epigenetic drivers with arrested development leading to oncogenesis. Lastly, clinical protocols and management have not caught up with novel molecular discoveries.

The neural stem cell divides into neuronal-restricted progenitor cells and glial-restricted progenitor cells. The neuronal-restricted progenitor cells divide into neurons giving rise to primitive neuroectodermal tumor, atypical teratoid rhabdoid tumor, and embryonal tumor with multilayered rosettes. The neural progenitor cells give rise to oligodendrocytes, and thus, oligodendroglioma. The glial-restricted progenitor cells subdivide further into oligodendrocytes, astrocytes, ependymal cells, giving rise to their respective brain tumors.

Previously, pediatric brain tumors were diagnosed histologically by neuropathologists by grading them 1 to 4 based on multiple criteria. The 2016 World Health Organization (WHO) still uses original criteria to classify brain tumors but adds layers for molecular and tissue-based information based on DNA methylation to uncover novel entities.

Low-grade gliomas arise in different brain and spinal cord locations and have different molecular alterations across the

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life span. Infant low-grade glioma occurs on average at age 3 and arises from the neocortex. These tumors are dominated by molecular fusions such as BRAF, MET, BRAFV600E, MET, and NTRK. Additionally, targeted inhibitors are now being used therapeutically in these tumors. Childhood low-grade glioma typically occurs in children ages 3-12 years old and originates in the posterior fossa. These tumors are controlled by MAPK alterations. Adolescent and young adult low-grade gliomas occur in those older than 15 years and originate in the hemispheres. Their growth is primarily driven by p53 alterations.

It was once thought that ependymomas were classified as a single disease, but multiple researchers have shown molecularly that there are at least 9 distinct subtypes, each of which could represent different therapeutic opportunities with targeted therapies.

Based on data generated by a collaborative research group, medulloblastoma now consists of at least 13 distinct molecular entities. Atypical teratoid rhabdoid tumor is a rare pediatric brain tumor, and there have been 3molecular subgroups identified. The genetic drivers in these subgroups are key to this tumor and may be important for developing novel therapies. Embryonal tumor with multilayered rosettes results from a fusion between TTYH1 and C19MC that leads to inhibition and, subsequently, overexpression of DNA methyltransferase.

In order to improve therapeutic options, there needs to be actionable targets in pediatric brain tumor subgroups. There should be a change in the clinical culture to have less rigid clinical trials and more flexibility that incorporates molecular diagnosis to aid in therapeutic management. Novel therapies are currently being discovered, such as kinase inhibitors, epigenetic modifiers, and immune modulators. The neuro-oncology community has seen a shifting practice from conventional therapeutic modalities (surgery, radiation therapy, and chemotherapy) to using more targeted and personalized therapies to help improve overall quality of life and survivorship.

Neuroimaging: Novel Investigations and Limitations

Kristen Yeom, MD; Lucille Packard Children's Hospital at Stanford University, Palo Alto, CA. Dr Kristin Yeom discussed novel imaging investigations and limitations of neuroimaging in pediatric brain tumors from 2007 until now. She began her talk by reviewing how in 2007, the last time NDC reviewed central nervous system tumors, neuroimaging viewed pediatric tumors on a macrostructural level to delineate their anatomy through improvements in image quality, contrast, and various modalities like diffusion-weighted imaging; however, imaging at the microstructural level was still limited. Another challenge at that time was the multiple molecular differences of pediatric tumors and how they appeared differently on imaging without further understanding of why it occurs in that manner. Since then, radiologic techniques have progressively become more advanced through understanding the molecular and biological differences of pediatric tumors through higher imaging resolution, improved capabilities to view microstructural levels, assessments using spectroscopy or physiologic parameters, and now through digital, computational ways to evaluate magnetic resonance imaging (MRI) and computed tomography (CT).

Before highlighting these advancements in pediatric tumor neuro-imaging, Dr Yeom discussed the challenges and limitations associated with imaging children. Imaging children poses unique challenges and limitations such as radiation exposure, imaging length, and persistent patient movement requiring sedation and coordination with anesthesia. Another challenge in imaging pediatric brain tumors are their difficult locations, especially with midline tumors like those in the orbits, sellar regions, or posterior fossa. These tumors are often hidden because of their location and masked by areas of soft tissue, bone, or air. The last challenge is the presence of multiple different tumor types with many different molecular differences. This can result in imaging differences even within a typical tumor subtype.

The next section of Dr Yeom's presentation focused on different imaging modalities within the last 10 years, starting with diffusion MRI techniques. Diffusion MRI is used to detect pediatric brain tumors in 2 ways. The first way is retrospective motion correction. In this modality, an EPI device with diffusion weighting and parallel imaging acquires images to restore brain sites that are prone to distortion, such as the orbital regions. These images are then reconstructed and complied on an outside data server, and then sent to the hospital for viewing. The next way in which diffusion tensor MRI imaging is useful is mapping motor and sensory pathway projections for surgical navigation and tumor classification. This is particularly useful for tumors in difficult locations, like optic pathway gliomas. It allows radiologists and the neuro-oncology team to advise neurosurgeons where the bulk of the tumor resides and help initiate alternative surgical approaches. This method can also be used to educate parents about their child's brain tumor through direct visualization. Lastly, these unique motor and sensory signatures can aid in classifying brain tumor subtypes based on cellularity and tumor pathology, potentially leading to better prognostic information to predict therapy response and survival.

Perfusion imaging has been used to classify brain gliomas in adult oncology but it has been challenging to use in children and had multiple limitations. The introduction of arterial spin labeling perfusion brought a suitable alternative to assist with characterization of pediatric brain tumors. Children have high intrinsic blood flow compared to adults, where increased signaling is delivered to the brain. This method does not require exogenous contrast, and instead, uses measurements of water molecules or blood at the level of the neck and brain. arterial spin labeling can be repeated if necessary and offers a direct quantitative method of perfusion. It is currently implemented in stroke imaging, assessing underlying vasculopathy, and now being applied to tumors. In tumor assessment, arterial spin labeling can be used to differentiate low-perfused versus high-perfused tumors and to better understand the underlying physiology and metabolism, leading to better imaging metrics to predict therapy response.

The last section of Dr Yeom's discussion focused on future imaging advancements, starting with the analysis of white matter fiber bundles. White matter fiber bundles enable a quantitative method to analyze how tumor pathways are shaped, deformed, and respond to therapy. Radiologists are also increasingly fusing metabolism and structural imaging together to analyze tumor structure and physiology. Iron oxide nanoparticles are also being uncovered for potential future use in pediatric tumor imaging. These particles act as strong intravascular binding agents in the blood vessels and macrophages, leading to potential uses for immune-based tumor imaging. Artificial intelligence and machine deep learning are rapidly accelerating advances in computing powers, processing speeds, and digital data throughout our lives, including in the medical field. This technology is not only useful for facial recognition and object detection, but also has potential benefits in pediatric neuro-oncology by extracting and mapping digital data to visualize pediatric brain tumors in a more mathematical way. By doing so, the tumor's complexity and geometry is retained and can be analyzed mathematically and statistically to potentially develop better models to map and classify tumors. This can also help cancer patients in other ways, such as better quantitative measurements of ventricles in cases of hydrocephalus. Overall, this technology provides a more volumetric, automated, and precise method for quantitative imaging. Dr Yeom continued by discussing the use of radiomics and individualized biomarkers to develop better prognostic indicators in pediatric brain tumors. Radiomics are defined as a field of medical study that aims to use large amounts of quantitative features from medical images using data-characterization algorithms. These radiomic features (edge, shape, texture, and histogram) have the potential to uncover characteristics that may not be detected by the human eye and allow better understanding of biological processes to be reflected in clinical imaging. It has the potential to uncover chemical signatures and pathways of different pediatric brain tumors and allow better subclassification, and possibly individualized therapy opportunities.

Many imaging methods have the potential to contribute to precision medicine, but there continues to be heterogeneity among pediatric brain tumors, and there needs to be validation and standardization of emerging quantitative biomarkers.

Neuropathology and Molecular (Re-)classification

Brent Orr, MD, PhD; St Jude Children's Research Hospital, Memphis, TN. Dr Brent Orr discussed the neuropathology and molecular classification of pediatric tumors. Tumor molecular classification is important, as it allows clinicians to prognosticate and predict how the tumor will behave clinically. Additionally, it allows therapeutic response prediction by homogenizing therapy groups and matching tumor types to effective therapies. Lastly, a classification system provides a framework for downstream basic and preclinical research to develop more accurate tumor models and study disease mechanisms.

The histopathologic classification of brain tumors is based on a histologic model developed in 1927 by Percival Bailey. This classification system was developed based on how the appearance of tumor cells related to normally developed tissues or cells. From the classification's installation in 1927 until 2016, there's been a steady increase in the number of tumor entities beginning at 13 classes to over 150. In the 1980s, there was a big jump from 78 to 127 classes, which corresponded to the development of antibodies in clinical practice. The increase in tumor entities was a result of multiple factors, such as the accumulation of new cases, increased knowledge of biology, and new advances in technology that allows for increased granularity of diagnosis. A recent increase in tumor entities is surging with the invention of next-generation sequencing and methylation profiling.

The historic histological model has multiple problems. First, the morphologic criteria were largely passed down through apprenticeship and were highly subjective based on clinical training. Second, cancer is extremely rare within the pediatric population and would be problematic for pathologists to develop experience when faced with these rare cases. Third, many common pediatric brain tumors share similar morphology and lack specific biomarkers. For instance, when comparing ependymoma, glioblastoma, atypical teratoid rhabdoid tumor, and medulloblastoma, all are hypercellular with a small cell phenotype and they are hard to differentiate from one another without additional analyses. Lastly, the histogenetic model does not account for molecular heterogeneity within individual diagnostic categories well. For example, medulloblastoma has 4 different morphologic subgroups via light microscopy; however more modern classification of these 4 subgroups (ie, WNT vs SHH vs group 3 vs group 4) cannot be distinguished via light microscopy.

Methylation profiling is changing the way pediatric neuro-oncology is understanding disease and molecular classification. It can be done through a variety of methods such as bisulfite DNA sequencing, but the most recent method is through methylation arrays developed by Illumina. Methylation arrays look at specific methylation sites (more than 850,000 sites), most notably CpG islands and RefSeq genes. This assay is very useful in clinical practice in that it does not require a lot of DNA and can viewed on a single slide, and it works just as well on the formalin-fixed, paraffin-embedded methylation as it does on frozen tissue. The 2 types of data received from the methylation assay is a specific methylation signature and copy number data resulting in distinct tumor types associated with stereotyped methylation sequencing. This represents a combination of tissue cell origin and driver mutation. For example, a heat map illustrating different tumor types demonstrates taking high-dimensional data, which is a CpG site feature, and conducting unsupervised cluster analysis to sort out the different tumors distinctly. The other type of data from methylation arrays is copy number data.

Dr Orr continued by discussing how to design a general methylation study. The design starts by developing a reference cohort (ie mutational data, copy number data, transcriptional data) of known tumor types, and then compare them to a test cohort by using orthogonal validation (ie, next-generation

sequencing or directed sequencing of fluorescent in situ hybridization, copy number profiling of fluorescent in situ hybridization, or RNAseq/transcription arrays). If the test samples cluster in the same molecular group, it would be interpreted as molecularly similar to the reference tumor. If the test samples distribute among different reference groups, then it would read as molecularly heterogenous. Lastly, if the test samples form a new group, then it would be interpreted as a novel molecular type.

There have been many examples of tumor classification where technology was used to modify the most recent 2016 WHO classification system. First, in the 2007 WHO classification system, gliomatosis cerebri was considered its own entity. This tumor is an infiltrative glioma involving 3 or more cerebral lobes. Gliomatosis cerebri is a rare disease, and therefore, there are limited molecular data and difficult to accumulate multiple cases and tissue specimens. In 2016, through methylation profiling and reference cohorts among other high-grade gliomas, it was discovered that gliomatosis cerebri is a heterogeneous tumor and a molecularly distinct tumor entity.

Another update to the 2016 WHO classification is the introduction of a tumor called embryonal tumor with multilayered rosettes. This is a tumor type where methylation forms a distinct cluster. It has a recurrent fusion with chromosome 19 micro-RNA cluster detected by either array, copy number, or by fluorescent in situ hybridization. Methylation profiling identified this single molecular tumor type along with variable histologic patterns and morphologies.

Another tumor type that has been affected by methylation profiling is the central nervous system primitive neuroectodermal tumor. Central nervous system primitive neuroectodermal tumor and its mimics are rare pediatric brain tumors, representing <3% to 5% of the tumor population. This type of tumor lacks specific biomarkers and well-defined histologic markings, which makes it very difficult for neuropathologists to diagnosis. Primitive neuroectodermal tumor often is indistinguishable from other high-grade gliomas that could impact therapies and clinical prognosis. In collaboration with the Cancer Research Center in Heidelberg, Germany, Dr Orr described research in differentiating the histologic patterns of central nervous system primitive neuroectodermal tumor. They took a large reference series of known high-grade tumor entities and compared them to institutionally diagnosed primitive neuroectodermal tumors. They found that 61% of primitive neuroectodermal tumors were clustered with the reference tumor entities, indicating that most primitive neuroectodermal tumors are misdiagnosed: 15% of tumors did not group into a specific cluster; 24% were grouped into 1 of 4 novel tumor types, and 1% were clustered with normal brain tissue, which could be a contaminant.[1] As a result of this study, primitive neuroectodermal tumor was removed from the 2016 WHO classification system. This study pointed to the power of the methylation assay to allow the discovery of rare and novel brain tumor types, such as central nervous system neuroblastoma with FOXR2 alteration, Ewing family of tumor with CIC alteration, high-grade neuroepithelial tumor with BCOR-TID, and high grade neuroepithelial tumor with MN1 alteration. Molecular profiling adds benefits to conventional test development and validation assays. For example, the primitive neuroectodermal tumor CIC tumors can be detected by immunohistochemistry (IHC) and fluorescent in situ hybridization. The BCOR tumor now have validated immunohistochemistry and polymerase chain reaction assays. And lastly, MN1 tumors can be detected using FISH. In a 2018 paper by Capper et al[2] in *Nature*, they developed a large reference series of brain tumors represented in the WHO. The researchers used a machine learning algorithm called the Random Forest to develop a supervised classification model. This allowed researchers to run a tumor test sample through this algorithm and give a compatibility score and class assignment.

The development of molecular methylation can change in how trials are designed. Dr Orr described a schematic for a clinical trial of medulloblastoma subgroups conducted at St. Jude's looking at molecular risk and adaptive strategy. Each molecular subgroup is stratified by methylation to expand each by a single biomarker to assess its risk and therapeutic response.

Problems previously described with the historic histogenetic model can be alleviated with modern approaches to classification. Methylation profiling is an objective measure and no longer requires subjective classification. Pediatric cancer is rare, but methylation allows accumulation of multiple tumor profiles to recluster data and classify rare tumor entities. Pediatric brain tumors lack specific biomarkers and have similar morphology, but methylation provides a versatile biomarker that generalizes across all tumor cell types. And lastly, methylation profiling is a powerful tool that allows for refinement of brain tumor classification and discovery of novel tumor types.

Significance and Limitations of Molecular Data

Scott Pomeroy, MD, PhD; Harvard Medical School, Boston Children's Hospital, Boston, MA. Dr Scott Pomeroy discussed the significance and limitations of molecular data in childhood brain tumors. It was previously assumed that every tumor was different, and to understand the heterogeneity, they were treated and followed over time. Some common elements were found to adjust therapies and increase survival rates and quality of life. For example, the standard risk of survival for medulloblastoma is 80%, but less than 10% diagnosed younger than 10 years old had a good quality of life after treatment. This fact demonstrated that the scientific community should not only increase precision therapies but also possibly decrease certain therapies with costly effects.

A study in 2015 by Quinn et al[3] showed the distribution of all primary pediatric brain and central nervous system tumors by histologic groupings. Over time the neuro-oncology research community has increased their precision on identifying and classifying pediatric brain tumors through the discovery of molecular classification and data.

Pilocytic astrocytoma is one of the major subtypes of low-grade glioma in children. Multiple molecular drivers contribute to one pathway, the MAPK pathway. A paper published by Jones et al[4] in 2013 showed the different molecular lesions that occur in pilocytic astrocytoma. The most common lesion is a fusion of KIA1549 that leads to an active *BRAF* gene in most cases. Current treatments focus on blocking signals downstream from *BRAF*, but may not consistently work all the time. Some tumors are responsive to this inhibitory mechanism and others are not. There could be multiple reasons for this. One reason is that blocking one downstream signaling pathway leads to an upregulation in another pathway that continues to drive tumor growth. There are multiple parallel pathways, but our framework of understanding is not complete yet. Biological systems are dynamic and respond to every action and reaction, but sometimes it is not the reaction we want.

Prior to molecular classification and identification of subgroups within medulloblastoma, it was previously divided into 2 categories: standard risk and high risk. If the age of diagnosis was older than 3 years old and the tumor was resected, these patients had a 5-year survival rate of 85%. Often, they were treated with craniospinal radiation and multidrug chemotherapy for about 1 year. The consequence of irradiating young children leads to a loss of up to 40 IQ points. These children may also suffer from cataracts, hearing loss, stunted growth, hormonal deficiencies, and possibly stroke.

The molecular era allowed the neuro-oncology community to view different tumors and transcription profiles to appreciate where tumors that may look similar had different gene expression signatures. It was previously believed that primitive neuroectodermal tumors were not just a single disease, but that sometimes it would act as a high-grade glioma and infiltrate the brain. Whereas at other times, it would be metastatic and act as an embryonal tumor. Differentiating between behaviors as a high-grade glioma and metastatic embryonal tumor makes a major difference when choosing treatment options.

Medulloblastoma has 4 subgroups: WNT, SHH, group 3, and group 4. The WNT subgroup is relatively homogenous and has a high incidence of beta-catenin 1 mutations. Overall survival is 95%. There currently is no specific targeted therapy, but clinical trial data dating from the early 2000s has suggested decreasing the craniospinal radiation to 1800 cG will still achieve the desired effect. Group 3 subgroup has a highly activated MYC pathway and in some cases amplification—these will have poorer prognosis with less than 50% survival.

The SHH subgroup is more heterogenous with p53 mutations, and characteristically has a poor prognosis. SHH is a very important molecule for the development of the cerebellum. It is secreted during development by the Purkinjie cell layer and aids in the development and proliferation of cerebellar granule cells. Prior to their migration from the external granule cell layer to the internal cell granule layer, the SHH binds to the PTCH receptor in its unbound state and inhibits (tonic) a transmembrane protein called Smoothin (smo). It then activates gene expression and modulates gene transcription. The problem here is that in many cases, the PTCH receptor is deleted or dislocated from the chromosome, which leads SMO to be tonically activated and drive the signal peripherally. The drugs currently used for SHH pathway mostly block SMO.

In 2014, Kool et al[5] superimposed driver mutations to see how it might block SMO in the SHH subgroup. They found that driver mutations, and in some cases, germline mutations, were actually downstream from SMO. A compound called SUFU is a common driver of this pathway, primarily in infants. Those molecular lesions and tumors are responsive to SMO.

Another caveat is superimposing another signaling pathway, such as mutations in p53, which are known to have a very poor prognosis. P53 is a DNA repair mechanism that has increased genetic instability. Even if treatments could block p53, its presence alone would indicate a poor prognosis for patients. Many current treatments operate by mutating DNA, like radiation and alkylation agents. These function to alter DNA structure, so treating a p53 tumor with these methods would only increase the tumor's genetic instability and prolong tumor growth. This is another important feature of why precision in molecular diagnosis is helpful.

There has been interest in developing drugs that act downstream and on transcription factors. The BET Bromodomain inhibitors have been developed, like JQ1, that act downstream of SMO. In cell culture, studies have shown that wild-type SMO tumor cells can be blocked by both SMO inhibitors and JQ1 downstream at the BET Bromodomain. When SMO is consistently active and not responsive to the hedge-hog inhibitor, it will be responsive to JQ1. It is quite promising in cell culture, but did not work well in animal models.

Within the landscape of medulloblastoma, the somatic mutation rate is quite low compared to adult cancers. Adult cancers have higher rates of mutations than pediatric cancers. Pediatric brain tumors have cells that are programmed to fall off the development pathway. The WNT subgroup is a very common mutation. For most other tumors, there are not a lot of mutations in one pathway, but a theme emerges among the various mechanisms. Many of these mutations affect the transcriptional apparatus. The genome is the driving force behind these tumors; however, the challenge occurs when targeting the transcription apparatus without targeting every cell of the body.

What do we do with all the molecular data? We can still create categories of high risk, intermediate risk, and very good risk. This is where the neuro-oncology field is heading now and in the foreseeable future.

Molecular Diagnosis in Embryonal, Glial, and Ependymal Tumors

David T.W. Jones, PhD; Cancer Research Center, Heidelberg, Germany. Dr David Jones discussed the molecular diagnosis in embryonal, glial, and ependymal tumors. The goal of molecular diagnostics is to develop an objective platform of tumor classification and provide an additional tool for neuropathologists in diagnosing. The discovery of molecular markers can provide further information to guide targeted therapies and prognosis. DNA methylation analysis operates by assessing

methylation of cytosine bases in a CpG context to provide information of epigenetic markers regulating cellular transcription during cell differentiation. During brain development, it is these epigenetic markers that are changing and modifying to adapt to cellular states throughout the development of different cellular lineages. In the molecular diagnostic context, there is evidence that DNA methylation enables better classification by acting as a surrogate marker for tumor cell origin. It is thought that tumor cells are a developmental expansion of a specific cell of origin and then maintains that epigenetic footprint where that cell undergoes transformation. Thus, the epigenetic marker acts as a surrogate marker of tumor cell origin rather than focusing on the activity of certain genes. This allows us to understand why there are so many different types of childhood brain tumors that are not seen in adulthood. There are many different cell types that present in specifically defined spatial and temporal windows during development, and many of these cells have the potential to give rise to a tumor when subjected to the right genetic differentiation. Once that time frame has passed, those precursor cells are not present anymore, and that tumor does not develop later in life.

The Cancer Research Center in Heidelberg, Germany, has been using DNA methylation analysis to provide objective analysis of pediatric brain tumors. They have shown that methylation analysis can reliably distinguish between 82 different central nervous system tumor types across ages and locations. Routine clinical use of DNA methylation leads to a reclassification rate of 10% to 15% of pediatric brain tumors.

Ependymomas are tumors of the ventricular linings and represent about 5% of pediatric brain tumors. These tumors have a highly variable clinical course ranging from benign after surgical excision to being refractory despite treatment. Ependymomas are divided into 3 major groups based on location—supratentorial, posterior fossa, and spinal—and then further subdivided into 9 subgroups based on tumor location and biological subtypes. Supratentorial ependymomas are driven by distinct molecular drivers, specifically genetic fusions. There are 2 subgroups of fusion mutations associated with supratentorial ependymoma. The first is C11orf95, which is the most common fusion. There is a structural change where the C11 protein is fused with a transcription activation domain. The C11orf95 fusion group has the worse clinical outcome. The second genetic fusion is the *YAP1* oncogene. This is coupled with a variety of different partner genes to create a structural protein that is similar to the protein domains maintained in the YAP1 fusion protein.

The analysis of posterior fossa ependymoma (PF) has been less clear in discovering the molecular driver events. There are 2 subgroups of posterior fossa ependymoma: PF-A and PF-B. Molecular diagnostics has shown that staining for trimethylation of H3K27 is a powerful surrogate marker for distinguishing between these 2 subgroups of PF. Within the PF-A subgroup, there is a complete loss of the histone trimethylation signal, whereas the vast majority of PF-B maintains that signal marker. This loss of histone trimethylation signaling is partly due to a gene called CXorf67 or EZHIP. Dr Jones described the work of Hubner in 2019 who discovered this connection between loss of histone trimethylation and CXorf67. CXorf67 is a naturally occurring gene protein within the body that closely mimics the structure of a histone that has methionine in position 27, replacing lysine. This mimicry is overexpressed in the PF-A subgroup of ependymomas. Overexpression of this protein inhibits EZH2 function and produces the same phenotype of loss of histone trimethylation. Identifying these molecular subgroups provide highly valuable information for the prognostic outcome of patients. Molecular subtyping in posterior fossa and supratentorial ependymomas is better to distinguish between outcomes in subtypes rather than the standard histologic grading process.

One of the tumors where quality of life is extremely important is in low-grade glioma. It is the most common cause of pediatric brain tumors, with approximately one-third of diagnoses. It generally has a good prognosis but can become a long-term chronic disease that recurs over a child's life and have damaging consequences from the tumor and treatment. Most of these tumors are driven by the MAPK pathway. An array of targeted therapies have been developed to inhibit this pathway downstream, such as MEK inhibitors and BRAF fusion inhibitors. Multiple studies have shown that low-grade gliomas can be split into biologically meaningful groups according to their methylation profile. Large pediatric low-grade glioma cohorts have been given firmer molecular footing for some rare tumors and identified possible tumor treatment targets. An example of this is the rosette-forming glioneuronal tumor. This tumor was previously recognized histologically but did not have a clear biological order to confirm diagnosis. When analyzing the DNA methylation profile of these tumors, they form a distinct group that is different from other pediatric low-grade gliomas. Rosette-forming glioneuronal tumor does not exhibit the usual single MAPK alteration, but instead has additional point mutations in FGFR1 gene and one-half have a second point mutation in PI3 K. This is a rare example of a low-grade glioma tumor that has a combination of 2 different pathways that need to be activated to form a tumor, even though there has not been evidence that these mutations worsen prognosis. Rosette-forming glioneuronal tumor clinically behaves like a low-grade glioma, but the MAPK alteration alone does not need to be activated for the tumor to occur. Another tumor associated with mutations in FGFR1 is extraventricular neurocytoma. This tumor is mostly seen in adolescents and young adults. The FGFR1 mutation is due to gene fusion, but also may have recurrent alterations. A large fraction of tumors that were histologically diagnosed as extraventricular neurocytoma but molecularly now belong to a different tumor type. A final example from the low-grade glioma spectrum is diffuse leptomeningeal glioneuronal tumor. This tumor entity was introduced into the WHO in 2016. Molecular analysis showed that not all these tumors show leptomeningeal spread, and thus its name may be a misnomer. Diffuse leptomeningeal glioneuronal tumor show KIAA1549: BRAF fusion and 1p loss. There are 2 distinct molecular subtypes of this disease: type 1 is seen in younger patients and type 2 is in older patients. These

subtypes are similar histologically and molecularly but can be separated via clinical course and overall survival rates.

High-grade gliomas are the most malignant pediatric brain tumors with median survival rates of 12-18 months. High-grade gliomas are classified by recurrent H3 mutations, specifically at sites of K27 M and G34R/B. These subsets are fundamentally different biological entities defined by their molecular mutations. Within the remaining histone fraction of pediatric high-grade gliomas, heterogeneity is still present and represents multiple molecular mutations such as alterations in PDGFRA, alterations in EGFR, and amplification of MYCN. Each of these mutations have clinical significance in terms of outcomes. In contrast to adult high-grade gliomas, where EGFR amplification is very common, pediatric patients showing EGFR alteration have better clinical prognosis than other subtypes.

There are new molecular groups that DNA methylation profiling helped discover. The Cancer Research Center in Heidelberg, Germany, established very large cohorts of tumors to profile through DNA methylation. They collected profiling data from more than 50,000 tumors. An example of their findings is a subset of K27 tumors that have additional low-grade glioma–like MAPK alteration mutation like BRAF, FGFR1, and KRAS. Histologically these tumors resemble low-grade gliomas and will initially behave as such but after several years may progress to histology resembling high-grade gliomas. The tumor will still harbor a combination of 3 mutations, but the overall copy number data of alterations will increase dramatically. This molecular and clinical change potentially alters therapy options and patient's survival rate. Another example are the tumors exhibiting non-K27, ACVR1 only mutations. These tumors have the same overexpressed genes as seen in ependymomas that lose K27 trimethylation. Expression data show increased CXorf67/EZHIP expression, as seen in PF-A, as an alternative mechanism of reducing H3K27.

The clinical impact of molecular neuropathology and molecular profiling is currently in practice and being prospectively assessed in the Molecular Neuro-pathology (MNP) 2.0 study. This is a collaborative study between researchers in Germany, Switzerland, and Australia. The goal is to provide DNA methylation profiling and gene panel sequencing from tumor and blood samples for all pediatric brain tumors. The MNP 2.0 collaborative has recruited approximately 350 to 400 pediatric brain tumor cases, with 80% of them in Germany; 1500 samples have been received. The data are collected to have a reference database to compare histologic diagnosis and biological workup, and to assess if there are any discrepancies leading to overall diagnosis. If a diagnosis discrepancy is found, the center arranges a discussion between the treating physician and the local center to discuss possible reasons for the discrepancy. The most common discrepancy observed was histologic high-grade gliomas, with molecular diagnosis consistent with low-grade glioma. This discrepancy could possibly represent an overgrading of pediatric brain tumors and leads to differences in therapeutic treatment. The MNP 2.0 is still a very new version of methylation classification with gradually increasing number of cases to 6000 and 172 molecular subclasses as a reference cohort. This system introduces a layered system of family, class, and subclass to reduce complexity and focus on the most clinically relevant information.

Since the last neuro-oncology discussion at the Neurobiology of Disease in Children symposium in 2007, there have been major technological developments, such as next-generation sequencing in multiple flavors and introduction of microarrays. There have been countless advances in understanding the biology of disease including PI3 K pathway alterations, histone mutations, multiple novel fusions, and epigenetic mechanisms of oncogenesis, and several of these have been translated to clinical advances. However, the task remains to assess how new biological data can be clinically relevant and impactful.

Session II: Pathogenesis

Overview of Pathogenesis and Mouse Modeling

Robert Wechsler-Rena, PhD. Dr Robert Wechsler-Reya's discussion focused on providing an overview of animal modeling and how they improve the understanding and treatment of pediatric cancers. Historically, most preclinical data have come from long-term cell lines. These cells are patient-derived and grown on artificial media such as plastic or bovine serum. They are a selection of a small subset of cells and that do not represent the heterogeneity of the tumor, and thus, highly unlikely to predict success of a drug in a clinical trial. More sophisticated studies of these cell lines involve placing the tumor into the flank of a nude mouse with no immune system. This method of using different species and a heterotrophic site distorts interactions within the microenvironment. The mouse's lack of immune system prevents studying immune-modulation and immunotherapies.

Dr Wechsler-Reya continued by discussing approaches to modeling brain tumors in mice, starting with germline transgenic and knockout mice. One of the first models of pediatric brain tumors was the germline PTCH knockout mouse that was developed in Dr Matthew Scott's lab at Stanford in 1997. This model introduced a lac-7 gene into the PTCH locus to knockout both alleles. This created an embryonic lethal event as there were several defects in the neural tube and other structures in the mouse. However, if one copy of PTCH is knocked out, the mouse survives, and some develop tumors that resemble human medulloblastoma. This research showed a snapshot of tumorigenesis and further investigation of potential therapies. A disadvantage of this model was that penetrance was low and the latency was very long, so further studies would need a very large colony of mice to monitor and study.

Dr Wechsler-Reya's lab was the first to develop conditional *PTCH* knockout in 2008. They accomplished this by crossing 2 lines of mice: one mouse with the gene you wanted to disrupt and a second mouse that expressed an enzyme recombinase to cut the genes. The lab used a promoter to drive expression in granule precursor cells in both mice, and then cut the *PTCH* gene, leading to knockout mice that developed human

medulloblastoma. The deletion can be done embryonically or postnatally. Many research labs used this strategy to express or knock out genes in the nervous system. Dr Wechsler-Reya again highlighted a recent experiment from his lab. This research involved expressing a recombinase in a variety of progenitor promoters in the central nervous system and using it to activate the *MDACA* gene. This is achieved by deleting the stop signal to turn on the genes. The goal was for the animals to develop MYC driven medulloblastoma, but this was not the case. Instead, these animals developed a choroid plexus tumor: low-grade choroid plexus papillomas and high-grade choroid plexus carcinomas. The low-grade choroid plexus papilloma resulted from activating MYC, and high-grade choroid plexus carcinomas has deletion of the *p53* gene.

Another approach that is more versatile in creating knockout or gene overexpression is to deliver the genes in vivo to the animal. An example of this is the RCAS-TVA system using an avian retrovirus. TVA is a receptor that allows all cells to be infected with the avian retrovirus. It resides in chicken cells, but not mouse cells. This experiment involved injecting a mouse with the avian retrovirus with hopes of transgenically expressing the RCAS-TVA protein in a specific cell, like neuroprogenitors. Specific genes were then delivered to the mouse via this model. A model similar to this was done by Becher et al to develop a model for diffuse infiltrative pontine glioma (DIPG). His experiment involved taking a TVA-mouse with a flanked p53 gene and infecting those cells with histone-3-k27 mutant virus encoding platelet-derived growth factor and CRE with the goal to delete the p53 gene. The 3-hit combination generated a tumor with molecular characteristics of high-grade glioma. Additionally, Holland et al used this model in a mouse to create an ependymoma by using promoters to encode a fusion, putting it into a TVA-mouse, and producing a tumor. The use of in vivo genes in mouse modeling allowed researchers to study the biology of a specific tumor and identify mutation drivers.

Another version of in vivo gene delivery is electroporation of the gene into the ventricle of an embryo. Dr Nada Jabado et al[6] in 2017 demonstrated this method while injecting plasma coated with the histone mutant to knock out the *p53* gene and knock down the *ATRX* gene. These elements go into the ventricle. Electrodes are placed on either side of the ventricles. DNAse is negatively charged and drives itself into the parenchyma of the developing brain. Tumors will only develop if the elements are delivered to the right place at the right time. The result is tumors that resemble high-grade glioma.

Ex vivo gene manipulation involves taking progenitors from the developing cerebellum and injecting them with viruses that carry relevant oncogenes, and then transplanting those cells into another mouse. This approach is highly flexible because it allows researchers to take out any gene to see if it gives rise to a tumor. An example of this was from Dr Wechsler-Reya's lab in 2012 where they used viruses encoding MYC and a dominant negative form of p53. These viruses were then placed into the cerebellum of an adult animal who developed a tumor that resembled group 3 medulloblastoma.

The previously described animal models are very useful for researchers. First, they allow researchers to study the origins of specific tumor cells. SHH medulloblastoma arises from cerebellar granule neuron precursors. WNT medulloblastoma arises within the brainstem. Ependymoma resembles radial glia cells on a transcriptional level. Radial glia cells from different parts of the central nervous system resemble different forms of ependymomas.

Second, animal modeling allows researchers to discover oncogenic drivers. This search starts with cell sequencing. One example was by Dr Paul Northcutt, where a region on chromosome 9 was noted to be continuously targeted by chromosomal alterations in group 3 medulloblastomas. That specific region was narrowed to a gene locus called GFI1B. It showed the gene was being activated by enhancers due to the chromosomal alterations. Catherine Lee from Dr Wechsler-Reya's lab extended this research model to prove how it contributed to tumorigenesis in 2014. She developed and isolated stem cells from the cerebellum, infected them with retroviruses coding for MYC, and transplanted these cells into another mouse. The combination of MYC + GFI1B lead to tumors in 100% of mice—this credentialed GFI1B as an oncogene driving tumorigenesis. Another example of discovering oncogenic drivers is studying the receptor activation of tyrosine kinase (RTK) and activation of the SRC pathway in group 4 medulloblastoma. Remke and Ayrault et al[7] in 2018 hypothesized that activation of the RTK and SRC signaling would be important for tumorigenesis. They tested their hypothesis via in utero electroporation. Plasmas encoding the *SRC* gene and a dominant negative p53 form were electroporated into the brains of embryonic mice. These animals went on to develop tumors resembling group 4 medulloblastoma. The formation of these tumors was particularly dependent on preserving the p53 pathway.

The last way preclinical animal models helped researchers is by allowing them to identify and test new therapies. Pediatric cancer is quite rare, especially pediatric brain tumors, and the use of mice models could help prioritize medical therapies and place them into clinical trials. Many models have been used to identify therapy treatments for group 3 medulloblastoma. Dr Wechsler-Reya described a 2016 study[8] from his lab where they used tumor cells from a tumor-bearing mouse, placed them into multiple-welled plates containing 3000 compounds, and monitored for 48 hours for impending cell death. The PI3 K and histone acetylene inhibitors (HDAC) were the most potent compounds that worked synergistically to initiate tumor cell death. Individually, these components inhibit tumor growth, but together, kill the tumor cells. This discovery could possibly be used in clinical trials for patients with recurrent medulloblastoma and brainstem glioma. Another example comes from the discovery of the GFI1 oncogenic driver in group 3 medulloblastoma. GFI1 works as a transcription factor by recruiting chromatin-modifying enzymes, including a gene called *LSD1*. Animals treated with this drug have a dramatic reduction in tumor size. The tumors do not grow in the brain, but in the flank, as they do not cross the blood-brain barrier. The last example involves using animal models to analyze immuno-

oncology components. Dr Wechsler-Reya's lab recently submitted a paper highlighting this feature by showing that in tumors with MYC + p53 expression, there is a downregulation of class MHC I. If a cell does not have class MHC I, it will not be recognized by the immune system. They rescued these cells by treating with low doses of TNF. When in combination with a checkpoint inhibitor, TNF can eradicate all tumor cells. Currently clinical trials are underway to see if this will work in human patients.

Mouse models have a lot of benefit to researchers as previously described, but they still do not completely capture the complexity of human disease. These deficiencies may be complemented by using patient-derived orthotopic xenografts that are derived from human tumor cells. These cells grow in an in vivo microenvironment and not cultured for extended periods of time. They maintain the heterogeneity and complexity seen in human disease. In the future, it will be very important to incorporate other therapeutic approaches used in clinical practice into preclinical studies. This would include the use of surgery/radiation/chemotherapy and using humanized patient-derived orthotopic xenografts to test immunotherapy. Lastly, animal models may be used to assess metastatic/resistance/tumor recurrence and enhancing drug delivery to the brain.

Neoplastic Transformation

David H. Gutmann, MD, PhD, FAAN; Washington University School of Medicine, St Louis, MO. Dr David Gutmann discussed the sociobiological aspect of neoplastic transformation. He began his discussion by referencing a sociobiology book from 1975 by Edward Wilson that proposed the idea that the evolutionary mechanism underly social behaviors in insects affects how their societies assemble. In these social insect societies, selective pressures operate on the society leads to evolution of advantageous social behaviors to preserve the species. These societies have a division of labor with each insect operatively for a very specific function. There are overlapping generations, so one does not simply die out. They have constant communication through the secretion of substances, like pheromones, that allow them to work in a coordinated fashion. All these specialized behaviors ensure colony survival. The brain tumor ecosystem resembles social insect societies and exhibit many of the same features. There are specialized cells within the tumor populations like microglia, cancer stem cells, and T cells. The tumor cells have overlapping generations and are constantly cycling in and out. And lastly, like insect communities, tumor cells communicate through soluble factors, like chemokine, cytokines, and growth factors. These behaviors help ensure survival of the tumor cells at the expense of its host.

Neurofibromatosis 1 (NF1) is a genetic syndrome where children develop low-grade gliomas, typically along the optic pathway. The syndrome is caused by a germline mutation in the *NF1* gene. Within optic gliomas, nonneoplastic cells have a single mutation in the *NF1* gene; the neoplastic cells have 2 copies of the *NF1* gene that are either mutated or nonfunctional. Bajenaru et al[9] in 2002 and 2003 experimented with mouse models by conditionally inactivating both copies of the *NF1* gene in the punitive cell margins of the optic gliomas. These mice did not develop tumors. The basis of *NF1* gene mutation requires it to be germline, and the cancer cells need both NF1 copies inactivated. The mice in this model showed that by making changes to the neoplastic cell, coupled with a previous germline mutation, may not only account for tumor growth—a somatic mutation along the germline NF1 mutation in non-neoplastic cells could also account for glioma formation, and thus important for glioma biology.

Microglia are an innate immune system–like cell of the brain that is important for the brain's response to infection and inflammation. It is involved in the neuron remodeling and synaptic pruning in autism and neurodegenerative diseases. Microglia are essential elements for tumor cell growth. Tumor cells secrete chemokines that attract microglia to the forming tumor bed. If chemokines are blocked, migration of microglia to the tumor bed is dramatically reduced. Microglia sense the chemokine signal through a receptor called CX3CR1. Research from Pong[10] in 2013 and Guo[11] in 2019 showed that when microglia have one copy of this receptor, the mouse model will have an optic glioma with reduced expression of the chemokine receptor. Additionally, the advent of the tumor will be delayed by 1.5 months. Tumor cells recruit the microglia, which is necessary for tumor migration, but how do the microglia instruct the tumor cells to grow? Research by Daginakatte et al[12] in 2007 and 2008 showed that the use of minocycline will inactivate microglia and block tumor growth. If microglia are completely depleted in the brain, tumor growth may be decreased. Dr Gutmann further described collaborative research with Elaine Mardis, PhD, where they isolated microglia from mice with and without tumors and performed RNA sequencing to examine what is different between nonneoplastic optic nerve glia and neoplastic ones. The group discovered the factor most strongly implicated in the attraction of immune cells was a chemokine, CCLC5. When CCLC5 is added to the neoplastic cells, they grow faster, hence acting as a growth factor. If the tumor cells from mice with optic gliomas are placed into a mouse with an intact immune system, but lacking CCLC5, there is no tumor formation. CCLC5 is required for tumor cells to grow. Microglia are attracted to tumor cells via secretion of chemokines, and they are reprogrammed to make a growth factor that is necessary for cancer cell growth.

Microglia activation is the next aspect discussed by Dr Gutmann. He references a 2015 study with Y-H Chen[13] from *Cell Reports* where they generated low-grade glioma cancer stem cells in a mouse and recapitulated the tumors by putting them back into a mouse with a completely intact immune system. The mice used were athymic and lacked T-cell maturation. When tumor stem cells were placed into mice with an intact immune system with defected T cells, no tumors were formed. That discovery meant that T cells were required in some way to educate microglia to create a supportive environment for cancer cells. In addition, they found that microglia from a mouse without functional T cells do not appear normal and lack

CCLC5, the previously described growth factor. The T cells instruct the microglia to change their biology to make CCLC5 and promote tumor growth. T cells can transiently cross the blood-brain barrier and leave through the lymphatic system. If T cells from a normal mouse are put into contact with microglia from a new mouse without T cells, the microglia can be instructed to make CCLC5 through pheromones and secreting factors into the environment. Overall, there is a complex relationship between tumor cells, microglia, and T cells. T cells are required to teach microglia to provide a supportive environment. Blocking T cells from entering the brain or overall function slows tumor growth. Likewise, blocking cytokines the T cells make to educate the microglia also slows tumor growth. Understanding this circuit and appreciating that T cells come from the blood may show ways to consider future treatment, especially if T cells are naturally recruited in brain cancer.

Microglia also play a possible role as to why NF1 patients with vision loss require treatment. Boys and girls are equally likely to develop optic gliomas; however, more girls are treated. In 2014, Michael Fisher collected a large cohort of patients that showed that girls were more likely to lose vision if they had an anterior optic pathway glioma. Kelly Diggs-Andrews[14] in 2014 and Joseph Toonen[15] in 2017 used mouse models to figure out why girls lost vision more frequently than boys. The loss of vision came from losing retinal ganglion cells in the back of the eye that project the retinal fibers into the optic nerve and eventually into the brain. Boys have a small increase in the number of retinal ganglion cells; however, girls lose about one-half of them initially and then completely by 3-7 months of age. Another contributing factor to gender differences in NF1 vision loss is the difference in gonadal sex hormones. In the same experiment in 2017, Joseph Toonen discovered that if the estrogen from was removed from the female mice with optic gliomas, the number of dying retinal ganglion cells decreased; in addition, it further increased the number of retinal ganglion cells and restored the nerve fiber layer. The receptor for estrogen-beta is also expressed on microglia, and blocking that receptor on the microglia had the same effect as when estrogen was removed.

Dr Gutmann's discussion highlighted the cooperative neuroaxis between non-neoplastic and neoplastic cells that can be interrupted by multiple factors. Microglia and T cells are the cells most responsible in establishing and operating this neuroaxis. This knowledge provides opportunities for additional therapeutic options. We can adjust our strategies to prevent further vision decline by impairing the ability of these microglia to make neurotoxins. There is additional opportunity to create a series of mice with the same genetic background that have different germline and NF1 mutations, as it will allow researchers to change tumor properties such as onset, latency of formation, growth, and penetrance. These mice will then have biological variability similar to that of human patient profiles and provide an opportunity to use mice in preclinical platforms that more accurately reflect the diversity of clinical disease. It would allow harmony between preclinical and clinical trial designs and outcome measures, so the community yields more effective therapies.

Genetic Predisposition Syndromes and Epigenetics

Nada Jabado, MD, PhD; McGill University, Montreal, QC, Canada.
Dr Nada Jabado discussed cancer predisposition syndromes and epigenetic deregulation in pediatric brain tumors. The use of genetic sequencing is steadily uncovering more mutations associated with pediatric brain tumors and leading to the discovery of more cancer predisposition syndromes. These mutations were previously thought to be somatic, but they occur in the germline. Common predisposition cancer syndromes are Li-Fraumeni, NF-1, Von Hippel-Lindau. Emerging familial predisposition syndromes are caused by the Dicer-1 mutation, which can affect multiple body systems and occurs in childhood. Dicer-1 is a microRNA gene processor that regulates gene expression during specific times in development. If the cells are not processed in a specific manner, the gene expression will be completely altered. Dicer-1 provides mutations, and multiple tumors have alterations in this processing system. Central nervous system manifestations of Dicer-1 syndrome include pituitary blastoma, pineoblastoma, macrocephaly, and embryonal tumor with multi-layered rosettes–like infantile cerebellar tumor.

The knowledge and discovery of Dicer-1 and its associated syndrome suggests that genetic counseling should be more routine in clinical practice. Genetic counseling involves assessing the genome for potential mutations through genotyping the patient's genes, gene panel sequencing, or whole genome/exome sequencing. The results will then dictate recommendations of treatment and intervention. Protocols have been developed to assess and identify cancer earlier in children with predisposition syndromes to hopefully improve survival rates and quality of life. Dr Jabado described the McGill Interactive Pediatric Oncogenetic Guideline electronic health tool designed to assist medical providers in identifying which patients need to be referred for genetic workup of a predisposition cancer syndrome. This application contains information about 140 tumor specific algorithms of solid tumor, hematologic malignancies, and benign tumors. Clinician select information in the algorithm based on tumor features, patient's personal history, and family history.

Dr Jabado continued her presentation by discussing epigenetic deregulation in pediatric brain tumors. Histones are the most ancient, conserved evolutionary protein. Histone 3 and histone 4 form a dimer to enable compaction of DNA into the nucleus, forming a nucleosome. Histones allow cells to express our genetic information, and partitions it to be read appropriately. The histone 3 variants are very important for brain development. In 2012, Susie Baker's collaborative group identified recurrent somatic, heterozygous hotspot mutations in the histone 3 variant at 2 positions. The first is at Lysine27 (K27) that was changed to methionine in any histone 3 variant. The second is Glycine34 (G34) that was changed to either arginine or valine in histone 3.3. It was discovered that these variants were

both present, and required, for pediatric brain tumors. K27 primarily occurred in midline tumors and G34 in cortex tumors.

As stated previously, histones are important for brain development and histone 3.3 is the stepping stone for the developing brain. Pediatric and young adult astrocytomas are the developmental defects of 2 histone 3 variants: K27 and K36. K36 is an inactive mark and never coexists on the same histone with K27. Brain stem astrocytomas occur in ages 3-5 years and are associated with K27M/ACVR1 mutations. When it occurs in 5-7-year-olds, it is associated with K27-H3.3/p53 mutation. Cerebral cortex astrocytomas occur in 15- to 35-year-olds and are associated with either the K36 or G34/p53 mutation. Thalamic tumors occur in 7- to 12-year-olds and are associated with either K27/p53 or K27/FGFR mutation. FGFR is important in diencephalic development. Frontal lobe tumors occur in 17- to 50-year-olds and associated with IDH/p53 and SETD2 mutations, which is a lysine 36 methyltransferase. Histone 3 K27 also affects areas of the hindbrain and midbrain, leading to tumor subtypes like high-grade glioma, atypical teratoid rhabdoid tumors, group 3 and group 4 medulloblastoma, and group A posterior fossa ependymoma.

Mutations within the epigenome are difficult to target as the epigenome changes with time and development. Tumor cells age until they encounter a specific mutation or genetic driver that stalls their development. The goal in targeting therapies is to find the mechanism that will unlock tumor cells and force them to differentiate. Lysine 27 trimethylation is the mechanism with the capability to unlock tumor cells. This component is potent in differentiated cells and rare in stem cells. Mutations within tumor cells inhibit lysine 27 trimethylation and prevents differentiation. Another method to target K27 mutant tumor cells is viral mimicry. Researchers use DNA methylation to prime the tumor cells to signal the histones to release and express their endogenous retroviruses. This is continually repeated to target the tumor cell and initiate cell death.

Inter- and Intratumoral Heterogeneity Underlying Medulloblastoma and Other Embryonal Tumors

Paul Northcutt, PhD; St Jude Children's Research Hospital, Memphis, TN. Dr Paul Northcutt discussed the inter- and intrahumoral heterogeneity in medulloblastoma. Medulloblastoma is a grade IV tumor by WHO classification, primarily affecting 7- to 8-year-olds. Histologically, it appears as sheaths of small round blue cells. Treatment options include surgery, radiation, and chemotherapy. Survival rate at 5 years is 60% to 80% depending on the subgroup. The medulloblastoma spectrum is molecularly and clinically distinct, and currently divided into 4 subgroups: WNT, sonic hedgehog (SHH), group 3, and group 4.

Methylation classification transformed how we subdivide and molecularly annotate medulloblastoma. We have dissected additional heterogeneity in the subgroups, which are referred to as subtypes. Dr Northcutt referenced a previous study of his from 2017 where they profiled approximately 1250 primary medulloblastoma patients using DNA methylation platforms to further classify subtype heterogeneity. Based on the study, the WNT subgroup was homogenous and the SHH subgroup had some heterogeneity based on age of the patient. Group 3 and group 4 subgroups were more complex when divided separately, but also appeared to overlap with 15% to 20% being a non-WNT, non-SHH group.

Genomic sequencing of medulloblastomas allowed further understanding of the lesions, the driver mutational events, and how they occur in individual cell groups. Most medulloblastomas in large cohorts have clear mutational events in genes that modify chromatin, as well as transcription factors that turn genes on and off. This discovery demonstrated a fundamental pathogenic mechanism in the formation of medulloblastoma based on the downregulation of transcriptional factors.

SHH medulloblastoma has a lot of intratumoral heterogeneity and can be further divided into 4 distinct subtypes: alpha, beta, gamma, and delta. Alpha subtype is predominately in children or older adolescents. It is characterized by p53 mutations either germline or somatic, and is associated with an overall poor prognosis. Beta and gamma are primarily seen in infants, whereas delta affects adults.

Group 3 and group 4 medulloblastoma consist of 65% of medulloblastomas. From Dr Northcutt's previous 2017 study examining 1250 methylation profiles, 8 distinct subtypes were found between group 3 and group 4 medulloblastoma. The researchers analyzed the demographics, histologic subtypes, and genetics of each subtype. Subtype 1 is driven by GFI1 and GFIB oncogenes initiated by an enhancer hijacking event of their transcription factors. Subtypes 2 and 3 are highly enriched with MYC, which is their primary genetic marker. These subtypes are particularly aggressive. Subtype 4 had no obvious genetic lesions. Dr Northcutt did not mention subtypes 5 to 7. Subtype 8 is highly enriched with chromosome modifier regions.

This new information of clinical subtypes is now being integrating into the clinical setting. Dr Northcutt continued by referencing a current study from his home institution at St Jude's where they performed methylation profiling on 310 medulloblastoma patients. When the patients were grouped based on the current classification nomenclature, the study found that all the WNTs survived, group 3 had a lower survival rate of 60%, group 4 had a survival rate of 90% but seemed to relapse, and SHH were somewhere in between. When looking at the individual subtypes, specifically group 3, it was noted that the 5-year survival rate for subtype 2 was 55%, subtype 3 was 45%, and subtype 4 was 90%. This finding further demonstrated the clinical heterogeneity in a specific subgroup.

Part of the new technology emerging in this field is the ability to sequence large cohorts of tumors, sequence the individual tumor cells, and define the cellular hierarchies and origins. Dr Northcutt highlighted a 2019 study from Hovestadt et al[16] published in *Nature* where they applied single-cell RTC sequencing to 8000 tumor cells from 25 primary medulloblastoma tumors using Smart Cell 2 technology; 11 patient-derived xenografts from the represented subgroups were also obtained. A mouse model was used to sequence cerebellar involvement

by sequencing 80 000 single cells using the patient-derived orthotopic xenograft platform. The mouse model provided a reference atlas to compare information from the single cell sequencing of the primary tumor back to development with an attempt to identify cell types present in the individual tumors, as well as, potential cells of origin; 13 developmental time points were sequenced that lead to a total of 90 000 cells. This research led to the development of a simplified structure where they attempted to identify cell lineages using their own markers.

The WNT subgroup demonstrated a cellular hierarchy largely driven by mitotic progenitor cell populations. It gives rise to much more differentiated tumor cells and an undifferentiated post-mitotic population. SHH has a unidirectional trajectory from undifferentiated progenitor cells toward more differentiated cells. Group 3 tumors were largely undifferentiated and a majority were locked in a primitive stem cell–like state mostly attributable to MYC. There was a high expression of MYC in these cells, as well as MYC target genes. Group 4 were much more differentiated, mitotic, malignant, and further along the differentiation trajectory. The tumors that exhibited features of both group 3 and group 4 were a mix of undifferentiated and differentiated cells.

Establishing the developmental origin of tumor cell lines provided important information for future drug-targeted therapies. Using the data sets of single cell transcriptomics in previous mouse and human cells, researchers used computational biology to map the lineage of cell origin for individual cells in medulloblastoma subgroups. The SHH subgroup had a very high correlation with granule cell progenitors. WNT originated in the lower brain stem and resembled astrocyte cells. Group 3 subgroup cells are completely undifferentiated and locked into the primitive progenitor cell state. Group 4 subgroup tumors had a high correlation with glutamatergic and GABAergic cell lineages.

There are multiple cellular hierarchies within medulloblastoma tumor subgroups, and this has further implications and opportunities for targeted therapies. The next step is to apply single cell genomics to treated, metastatic, and relapsed tumor samples.

Session III: Therapy

Overview of Clinical and Translational Trials

Nicole Ullrich, MD, PhD; Boston Children's Hospital, Boston, MA. Dr Nicole Ullrich discussed the clinical and translational clinical trials associated with multiple pediatric brain tumors. She started her discussion with a brief overview of the previous presentations. Brain tumors are extremely diverse and there is a huge spectrum of histologic and molecular subtypes, each with markedly different growth rates. Pediatric brain tumor treatment largely depends on the tumor subtype and can have profound impact on morbidity and mortality. Signs and symptoms of the tumor are related to the anatomical locations.

There are many treatment options available to treat pediatric brain tumors. Maximal surgical tumor debulking is the initial treatment of choice and provides tissue for diagnosis and molecular characterization that is crucial in understanding tumors and tailoring their treatment. Additionally, if the tumor is benign, further therapy is not needed. Chemotherapy is often used before, during, and after surgery and radiation; however, in children, it is used to delay radiation. Radiation therapy is used for certain tumor types and if residual tumor is present after surgery. Long-term effects of radiation therapy include necrosis, vascular change, cognitive issues, hormonal deficits, and secondary tumors. Lastly, stem cell transplant is an option but it is not commonly used.

Dr Ullrich's discussion continued in a case-based fashion to describe common pediatric brain tumors. Case 1 presented was a 4-year-old boy with a 3- to 4-week history of vague headache and intermittent vomiting. He had no gait disturbances or visual complaints. CT scan revealed a left cerebellar mass, and MRI showed a contrast-enhancing cystic mass in the posterior fossa mostly consistent with low-grade glioma. Low-grade gliomas account for nearly half of childhood brain tumors with a 90% 5-year survival rate. Worse prognosis is associated with a midline location and subtotal resection. Low-grade gliomas are classified as WHO II and accounts for various subtypes that rarely progress to high-grade tumors. The V600E point mutation in BRAF has been identified in a variety of low-grade gliomas including pleomorphic xanthoastrocytoma (70%), gangliogliomа (20%), as well as WHO III ganglioglioma. Observation may be the primary treatment option; however, they can also be cured surgically. Juvenile pilocytic astrocytoma (JPA) is a common variant of low-grade glioma. It is classified as WHO I with well-differentiated slow growth. It generally does not demonstrate subarachnoid spread. Sixty percent to 80% of juvenile pilocytic astrocytoma have a BRAF duplication and associated with the activation of the MAPK pathway. Juvenile pilocytic astrocytoma is also the most common tumor associated with neurofibromatosis 1 (NF1). Again, observation may be the primary treatment option and can be surgically excised depending on the location. The guiding principle toward therapy should aim to minimize long-term tumor- and therapy-related morbidity, and this can be achieved through observation, surgery, and local control with radiation. Additional therapy can be with chemotherapy, specifically vincristine/carboplatin combination, or thioguanine, procarbazine, lomustine, and vincristine (TPCV). Patients may require more than 1 chemotherapy combination with the goal to control the tumor and tumor stability. Targeted molecular therapies are starting to include mTOR pathway and MEK inhibitors.

Case 2 presented was a 7-year-old boy with a history of ataxia, drooling, slurred speech, and difficulty swallowing, demonstrating with signs and symptoms of diffuse infiltrative pontine glioma. Diffuse infiltrative pontine glioma represents about 10% of all pediatric brain tumors. It starts in the brain stem and then diffusely infiltrates the rest of the brain. Its pathology is mostly consistent with astrocytomas grade 2-4 and classified as a poor prognosis regardless of WHO staging.

Median age of onset is 7 years old. MRI shows diffuse expansion into the pons and engulfment of the basilar artery. Despite the poor prognosis, there are some treatment options available to control tumor growth. Local radiation can be used and may increase survival from 2 to 11 months. Lastly, there are clinical trials of immunotherapy and infusion of radiolabeled antibodies. In a clinical trial of 1130 patients from Hoffman et al in the *Journal of Clinical Oncology*, 40% of patients lived longer than 1 year, 10% longer than 2 years, and less than 12% lived for 3 years; 20% to 30% of patients had metastases at the time of death.

Case 3 presented was a 12-year-old girl with a 2-month history of headaches and intermittent vomiting. She was treated in the emergency department for these symptoms and after several weeks developed gait changes and intractable vomiting. Her findings were consistent with high-grade glioma. High-grade glioma represents a minority of pediatric brain tumors and is classified as WHO grade III anaplastic astrocytoma or WHO grade IV glioblastoma multiforme; 5-year survival rates for these grades are 35% and 10%, respectively. Treatment options include combination of surgery, chemotherapy, radiation, and targeted molecular therapies focusing on H3K27, IDH, and p53.

Case 4 presented was a 12-year-old boy presenting with several weeks of intermittent vomiting, headaches, and ataxia representing ependymoma. Ependymoma represents between 6% and 12% of pediatric brain tumors and may develop across all age groups. This tumor arises within or along any site within the ventricular system like the ependymal lining or near the central spinal cord. An overwhelming majority are intracranial (90%), with a small amount in the spinal cord (10%). The survival rate depends on the extent of resection with worse prognosis if younger than 2 years. Treatment options depend on its extent based on MRI of the brain and spine and cerebrospinal fluid cytology. Options include surgery, conformal or proton radiation therapy to the tumor site, and chemotherapy. Emerging therapies include molecular therapies and experimental trials.

Case 5 presented was a 4-year-old girl with a history of speech delay presenting with a 1-month history of headache and vomiting. Her symptoms were initially attributed to gastroenteritis, but symptoms persisted and worsened. Her headaches continued and she underwent imaging that showed a medulloblastoma. Medulloblastoma accounts for 10% to 20% of all pediatric brain tumors. It predominately lies midline, arising from the cerebellar vermis and adjacent to the fourth ventricle near the brainstem. Most of these cases spread to the central nervous system at the time of diagnosis. Medulloblastoma can be divided into standard and high risk with a 70% to 80% and 60% to 70% 5-year survival rate, respectively. Standard therapy includes surgery, radiation therapy, and chemotherapy with vincristine/Cytoxan/cisplatin. High-dose chemotherapy with stem cell rescue is reserved for newly diagnosed infants, extremely high-risk patients, and recurrent tumors. Lastly, stem cell transplant and phase I chemotherapy may be used in those with relapsed tumors.

Case 6 presented was an 8-year-old boy with rapid visual deterioration and field cut, as well as increased thirst. He complained of headaches that were worse in the morning. Parents reported him increasingly irritable and participating less in school. Histology was consistent with craniopharyngioma. The peak age of onset of this tumor is 5-14 years of age and tumors are localized in the suprasellar area. Symptoms are usually nonspecific and resemble this case presentation with the additional inclusion of hormonal deficiencies, obesity, sleep disorders, memory recall deficits, and disorganization. At presentation, the best treatment options are surgery and possible radiation. If relapsed, patients can benefit from another resection and possibly from treatment with BRAF inhibitors, intracystic radioisotopes, or bleomycin. Craniopharyngiomas have a good overall survival rate, but poor morbidity especially when associated with panhypopituitarism, central obesity, neurocognitive dysfunction, or sleep apnea.

Case 7 presented was a 16-year-old male adolescent with new-onset diabetes insipidus whose mother reported him bumping into objects and having intractable nausea and vomiting. Additionally, case 8 noted a 7-year-old boy who had voice deepening, presence of pubic hair, weight gain, increased growth, and gonadal enlargement. Imaging was conducted in both cases and showed a mass suspicious for central nervous system germ cell tumor. Central nervous system germ cell tumors account for a minority of pediatric brain tumors and peak at 10-14 years of age. Most of them (80%) arise in structures along the third ventricle with two-thirds in the pineal and one-third in the suprasellar regions. Pineal region tumors are more common in male patients, whereas suprasellar region germ cell tumors are more common in female patients. These tumors may also locally spread to the spinal cord and spinal fluid. Treatment depends on the type of germ cell tumor: germinoma vs nongerminomatous germ cell tumor. Germinoma treatment includes radiation with possible adjuvant chemotherapy for reduced radiation doses. Nongerminomatous germ cell tumor treatment is more sensitive to chemotherapy, with radiation less frequently used.

Dr Ullrich concluded her discussion by highlighting the future of pediatric brain tumor treatment. Future treatment options in clinical trials include novel drugs, antiangiogenic therapy, gene therapy, immunotherapy, and radiation sensitizers. Novel delivery systems include convection therapy, intrathecal chemotherapy, implantation of wafer and beads, and photodynamic therapy.

Targeted Therapies

Roger Packer, MD; Brain Tumor Institute, Children's National Medical Center, Washington, DC. Dr Roger Packer discussed novel targeted therapies in treatment of pediatric brain tumors. His discussion started with an overview of treatment approaches. Targeted therapies offer the opportunity for personalized precision medicine. They can be more selective than chemotherapy or radiotherapy, more effective, and have less side effects; however, some questions do arise with their use.

For instance, what molecule will be targeted and how to best measure efficacy?

Targeted therapies are used to target a single mutation, cellular pathway, and epigenetic modifications. It has been used in the adult neuro-oncology since the 1990s, with initial therapies focused on the overexpression and later amplification of receptor tyrosine kinases. In pediatric neuro-oncology, initial targets were also receptor tyrosine kinases, especially epidermal growth factor and platelet-derived growth factor; however, the initial experience was quite disappointing. These therapies were used as single agents and used in cases of recurrent tumors. They were not used in enriched pediatric tumor populations, not biologically driven, and there was concern if the therapy was at all effective.

The new era of pediatric tumor therapy is focused on targeting oncogenic fusions, such as BRAF. There are ongoing trials with small molecular inhibitors that bind and/or occupy the receptor site or the use of monoclonal antibodies to bind to the receptors. If these agents were to be successful, they should be tested and used in a biologically enriched population as part of a cocktail of multiple molecularly targeted agents.

The first targeted therapy discussed was vascular endothelial growth factor and antiangiogenic therapy. The vascular endothelial growth factor family has 5 related glycoproteins critical to wound healing. It promotes tumor growth, increases tumor migration, and initiates endothelial cell invasion.

Bevacizumab is a humanized monoclonal antibody that inhibits vascular endothelial growth factor. It is currently approved for the treatment of adult glioblastoma multiform. This agent does not cross the blood-brain barrier. In the Herby trial by Gill et al[17] in 2018, bevacizumab, coupled with temozolomide failed to provide a survival advantage for pediatric high-grade gliomas during postradiation therapy; however, it does have some benefit in pediatric low-grade gliomas when paired with irinotecan. Irinotecan is an analog to camptothecin and a pro-drug to a topoisomerase I inhibitor. It has been approved for multiple adult cancers with primary toxicity of gastrointestinal side effects. Dr Packer recounted an index case using bevacizumab and irinotecan in an 8-year-old boy who presented with blindness and weight loss for 6 months. The patient failed multiple chemotherapies and radiation therapy and eventually was placed on this treatment combination as a last resort. After the first cycle of treatment over a 2-month period, the tumor shrank and the child regained vision. Early experiences with this therapy combination have shown benefit in up to 80% of pediatric patients with low-grade glioma, particularly in neurologic improvements and decrease in tumor size. One consequence to using this therapy is that after discontinuation, the tumor returns or increases in size; however, the neurologic deficits do not return. This index case, and others, have led to ongoing randomized phase 2 clinical trials.

Targeted therapies function to target multiple areas of the cell including downstream signal transduction, stem cells, epigenetic changes, and p53.

The first molecular target of a signaling pathway that was targeted was rapamycin in patients with tuberous sclerosis complex. Rapamycin targets the mTOR pathway and causes regression of astrocytomas in patients of this cohort, resulting in decreased tumor size. Dr Packer recalled previous studies that also showed improvements in seizure control, halted progression of the disease, and improved the patient's intellect.

Another agent used to inhibit the mTOR pathway is everolimus. This has been used in NF1 patients with low-grade gliomas. Current phase 2 studies suggest a partial response in 4 of 25 patients monitored over 1 year and that it stabilizes tumor progression.

A major breakthrough for molecular targeted therapy for pediatric brain tumors has been using the BRAF gene fusion duplication in pilocytic astrocytomas. Multiple labs, including those by Stefan Pfister and David Jones have worked on its discovery and continued effects. The discovery of BRAF fusion duplication provided the first real molecular target using a single pathway mutation.

MEK inhibition is a downstream signaling pathway target that may also be used for pediatric brain tumor therapy, more specifically in low-grade gliomas in patients with NF1. The first drug being tested is selumetinib which is a selective, non-ATP competitive small molecule inhibitor of the MAP/MEK pathway. A phase I trial by the Pediatric Brain Tumor Consortium in April 2013 studied the potential toxicities of this drug in 38 subjects who had low-grade glioma and failed either chemotherapy or radiation therapy. About 50% of subjects had a partial response and 25% to 30% had a complete response. Common toxicities included rash, mucositis, and elevated amylase and lipase. Additional studies included a phase 2 trial assessing the durability of therapy response by comparing subjects with low-grade glioma with NF1 and those with BRAF fusion mutation—25 patients were collected in each grouping. In the NF1 subgroup, 16 of 25 patients completed all 26 courses of the drug and 40% of them had a 50% reduction in tumor size. Patients were off treatment for about 3 years and 5 had to be retreated. In the BRAF fusion mutation subgroup, 14 of 25 completed all 26 courses of the drug and 70% of them had a 50% reduction in tumor size. Patients were off treatment for about 3 years as well, and 7 had to be retreated. Future trials with MEK inhibitor drugs will lead to combinations with chemotherapy, hydrochloroquine, and mTOR agents.

Medulloblastoma is a molecularly heterogenous tumor entity with multiple subgroups and sub-typings. The subgroups are divided into WNT, sonic hedgehog (SHH), group 3, and group 4. Subtypes are further divided among these subgroups. The SHH subgroup has been the primary target of developing novel target therapies. It has 2 therapeutic subgroups that are mutation driven: (1) upstream mutations primarily seen in infants and adolescents and (2) downstream mutations. As was previously discussed in preceding lectures, SHH pathway is critical in embryogenesis of the neural tube and other organ development, as well as cerebellar development, particularly granule neuron progenitor cells. Clinical trials published by Robinson et al[18] in the *Journal of Clinical Oncology* in 2015 assessed the use of vismodegib in treating SHH medulloblastoma. The trial assessed 31 adults and 12 children and their

response to the drug. In children, this drug caused growth plate damage through premature fusion of the growth plates and resulted in premature closure and severe short stature.

The NCI-COG pediatric MATCH study is currently undergoing a single-stage phase 2 clinical trial of genomically directed therapies for children with refractory solid tumors and lymphomas. The clinical trial is assessing genetic sequencing and mutations in multiple targets to select a study agent and, hopefully, assess patient's response to drug treatment.

Radiotherapy

Tom Merchant, DO, PhD; St Jude Children's Research Hospital, Memphis, TN. Dr Tom Merchant presented advancements in radiation therapy in the treatment of pediatric brain tumors. Pediatric radiation oncology practice occurs in 3 steps. First is assessing the patient's indication for treatment and sequencing of treatment options. Second is treatment planning through imaging (CT and MRI) and choosing optimal radiation dose and volume. Lastly, there is the actual treatment that is modified based on tumor characteristics and the patient's response.

Medulloblastoma treatment primarily consists of surgery, craniospinal radiation at 36G, and posterior fossa boost at 54G. The substantial radiation to the brain often causes poor cognitive outcomes, with approximate loss of 3.7 IQ points per year (Walter et al,[19] *Journal of Clinical Oncology*, 1999). Early clinical trials in the late 1990s researched the effects of reducing the craniospinal dose versus reducing primary target site volume. Thomas et al[20] in 2000 showed that lowering the craniospinal dose to 23.4G was associated with an increased risk of neuroaxis tumor recurrence. Packer et al[21,22] in 1999 and 2006 combined chemotherapy with reduced craniospinal radiation dose of 23.4G and achieved an 82% to 85% 5-year survival rate. Walter[19] in 1999 and Ris et al in 2001 demonstrated that reducing craniospinal radiation from 36G to 23.4G alone does not impact IQ decline. Dr Merchant described his own study from 1999 that assessed reducing the primary target to only the tumor bed with a limited margin and decreased the amount of high dose radiation. This research showed favorable outcomes as well. Dr Merchant continued by discussing the SJMB96 (1996-2003) and SJMB03 (2003-2013) protocols from St Jude's Hospital. These protocols focused on medulloblastoma patients and examined the typical treatment regimen of surgery, craniospinal radiation therapy, and chemotherapy. The protocols attempted to reduce the amount of radiation to the brain by using formal radiation therapy methods and decreasing the volume. The first protocol reduced radiation dose to the brain by 50% and the second protocol reduced it by 30%. Improvements in IQ decline were also seen with an average of 2.4 points per year.

The Children's Oncology Group from 2004-2014 further assessed decreasing radiation dose to 18G in medulloblastoma patients younger than 8 years. Patients were divided between 18G and 23.4G craniospinal radiation, randomized, received 54G radiation to primary site or posterior fossa boost, and lastly received 9 cycles of chemotherapy. Early results of this study showed they could not treat the entire posterior fossa, but only the tumor bed within a limited margin. There was an equivalent outcome between these groups. Decreasing the radiation dose to 18G was unsuccessful in both groups, and there was increased risk of recurrence.

Infant medulloblastoma (children younger than 3 years) was typically treated with chemotherapy. Radiation therapy was deferred unless there was tumor recurrence. Unfortunately, most of these patients would relapse within 6 months of chemotherapy treatment and underwent craniospinal radiation. The Children's Oncology Group P9934 Protocol (2000-2006) piloted a study looking at this subset of medulloblastoma patients. They analyzed patients who underwent 4 cycles of postsurgical chemotherapy and focal irradiation to the tumor bed. This improved tumor control rates in any portion of the brain that achieved 12-15G of radiation.

Lastly, medulloblastoma is a highly diverse and heterogenous tumor with multiple subgroups and differences in treatment. The SJMB12 study analyzed 8 different treatment regimens among the medulloblastoma subgroups. WNT medulloblastoma had 100% event-free survival and no failure at 23.4G craniospinal radiation. The SHH patients are a mix of 40% to 75% event-free survival. The non-WNT/SHH patients have a 55% to 85% event-free survival. The current craniospinal dose is 15G, with 51G at the primary target site. 23.4G is used in intermediate/standard-risk patients, and high-dose craniospinal radiation is used in those with more adverse prognostic features.

The advent of new technology and proton beam therapy has allowed more focal treatment of medulloblastoma. Radiation oncologists carve out the cranial subarachnoid space and direct proton beams in specific areas to achieve larger coverage while minimizing side effects. Proton beam therapy decreases therapy dose to extra–central nervous system tissue and has the most success in preserving patient's IQ.

Ependymomas are subdivided based on their location. Infratentorial ependymomas are associated with neurovascular structures. Supratentorial ependymomas are associated with larger tumors that impede surrounding structures and require very large treatment volumes. Many of these patients are younger than 3 years at time of diagnosis and are particularly challenging for radiation oncologists. Historically, the prognostic outcome for children with ependymomas were quite poor. Children who underwent surgery, craniospinal radiation therapy, and postsurgical chemotherapy had a survival rate of 40%. If radiation therapy was deferred until tumor relapse, the survival rate was worse. Recent advances in surgery and radiation therapy have improved survival outcomes in children with ependymomas. In 2009, Dr Merchant and colleagues published results from the St Jude Children's Research Hospital RT1 Protocol that documented results of 150 children with ependymomas. There was excellent local control of 83%, overall survival rate of 81%, and event-free survival rate of 72% at 5 years. Radiation therapy methods evolved throughout the years and included the use of proton therapy. Intensity-

modulated proton therapy allows radiation oncologists to carve out critical structures in the brain to minimize toxicity.

The Children's Oncology Group study from 2003-2007 analyzed children with ependymoma older and younger than 3 years old. The <3-year-old group also included children as young as 12 months. The study analyzed outcomes in these children as they underwent surgery, chemotherapy, and post-surgical radiation therapy and found equivalent treatment outcomes between the groups, provided treatments were the same. The event-free survival rate almost tripled in older children who received immediate postoperative radiation in comparison to those that received chemotherapy and deferred radiation therapy. Another aspect of this study was the prognostic factor of the group A posterior fossa ependymoma subtype, which typically has a poor prognosis. There was no statistical difference in prognosis in children who received immediate postoperative radiation.

Central nervous system germ cell tumors have complex geometry and are really challenging for radiation oncologists. In germinomas, researchers have used chemotherapy to reduce the dose of radiation and target the ventricular volume with doses as low as 18G and 30G at the primary site. This treatment has been quite successful in survival outcomes with low concern for neuro-axis dissemination. Dr Merchant described another study in 2004-2008 concerning nongerminomatous germ cell tumors where they analyzed patients who underwent surgery, 6 cycles of chemotherapy, and craniospinal radiation at 36G and 54G at the primary site. This resulted in a 90% survival rate at 5 years. The 2012-2018 study analyzing the same tumor type had the same surgical strategy and chemotherapy but included ventricular radiation at 30G and 54G at the primary site—there was no craniospinal radiation. The event-free survival rate was less than 80%. Future treatments would involve irradiating the ventricles and spine, but the potential for brain metastasis in nonirradiated areas exists.

Radiation therapy is a good strategy for low-grade glioma including diencephalic and optic pathway gliomas. Studies in 2009 and 2019 showed better tumor control and visual outcomes in grade I and II tumors with smaller clinical targets. Hippocampal-sparing proton therapy may also be used to improve outcomes in low-grade glioma patients. There is a dose-dependent relationship to this method, and clinical trials are currently underway.

The primary treatments for craniopharyngioma is surgery and radiation therapy. Boehling et al[23] in 2012 assessed the utility of reducing radiation dose to normal tissues by using proton versus photon beam therapy. The limited surgery and proton therapy trials in 2011-2016 and 2019 showed equivalent disease control between proton and photon therapy treatments. There were almost equivalent decreased rates of necrosis and severe vasculopathy. Additionally, there was improved cognitive outcomes in those who received a reduced volume of therapy at low and intermediate doses.

Advanced radiation modalities include stereotactic radiosurgery like gamma knife, cyber knife, and Linac-based SRS. The gamma knife and cyber knife technologies are methods of external beam radiotherapy that accurately delivers a high dose of irradiation in 1 to 5 fractions to a cranial target. These methods are minimally noninvasive and spare normal tissue but should be used selectively in children. MRI/Linac allows practitioners to image a patient before each treatment to develop individualized treatment plans. Robust analysis is another advanced method used to deliver individualized treatment plans. It involves using software algorithms to analyze treated target volumes with radiation therapy.

Radiotherapy fails for a variety of reasons including the inability to detect active disease that would have benefited from higher-dose treatment and/or surgery. Second, radiotherapy is not yet customized to true risk of recurrence. Third, localization and verification require newer technological methods that are yet developed or deployed. Finally, radiotherapy can be associated with multiple side effects.

Immunotherapy

Eugene Hwang, MD; Center for Cancer and Blood Disorders, Children's National Medical Center, Washington, DC. Dr Eugene Hwang discussed immunotherapy techniques for treatment of pediatric brain tumors. Previous studies in adult melanoma and leukemia have shown that the adaptive immune response can kill cancer cells effectively, and this is promising for the immunotherapy field. The adaptive immune system operates through a specific pathway involving cancer antigen presentation, priming and activation, and signaling T cells to infiltrate the tumors—all leading to cancer cell death. The immune system has a cellular balancing mechanism in which there are activating and suppressor factors that modulate its response. Activating, or effector, cells are used to make the immune cells strong to combat the cancer cells. Examples are cytotoxic CD8 T cells, dendritic cells to present the antigen and initiate T-cell activation, and natural killer (NK) cells. The suppressor cell mechanisms enable tumor cell proliferation by inhibiting the body's protective responses. Examples are regulatory T cells that inhibit effector B and T cells, myeloid-derived suppressor cells that inhibit T-cell activation, and inhibitory macrophages. There are additional checkpoint inhibitors (ie, TCR, CD28, CD40 L, CTLA-1, PD-1) that line T cells to deactivate an antigenic cell and make it invisible to the immune system. Within the tumor itself lies a complex microenvironment with a vast heterogeneity of antigens expressed, linked, and regulated by the tumor cells. The complexity of the immune system poses difficulty in designing effective immunotherapies to combat tumor cells because it requires precise identification of neoantigens and neoepitopes.

The central nervous system was previously described as "immune privileged" and invisible to the immune system. It was thought to lack lymphatic drainage, had few antigen-presenting cells (APCs) with low MHC expression, and primarily consisted of a blood-brain barrier of proteins and cellular transport. Modern technology allowed researchers to discover that the central nervous system is in fact not invisible and is quite complex and specialized. Cerebrospinal fluid drains

through the olfactory bulb to cervical lymph nodes, mediated by antigen trafficking. The blood-brain barrier can be disrupted by tumor cells. T-cells have a robust mechanism that transports them through the blood-brain barrier to targeted areas. There is also a large concentration of activated T cells in the cerebrospinal fluid with multiple molecular targets (ie, VLA-4, LFA-1, and CAR T cells). This new model will hopefully lead to adjustments in immunotherapy to better target tumor cells.

There are multiple immunotherapy techniques currently underway for pediatric brain cancer treatment. Passive antibodies bind to surface antigen on the tumor surface to enable biologic modification. They bind long enough to initiate effector cell mechanisms and prevent internalization. An example of passive antibodies is BITE. This mechanism allows binding of T cells and tumor cells. It is more versatile, safe, and inexpensive, and does not require stimulatory factors. It functions by converting regulatory T cells into cytotoxic T cells to initiate cell death. An example of BITE is blinatumomab, which has been highly successful in B-cell malignancies.

Adoptive cellular transfer (ACT) is another immunotherapy technique that involves T-cell harvest and chimeric antigen receptors (CAR). T-cell harvest involves harvesting T cells from a patient, linking them to specific tumor antigens, and reinfusing them into the patient to illicit immune response. This method is highly individualized, and the durability is unknown. CAR involves using fusion genes to link nonspecific T cells to tumor antigens. Preclinical data have been promising for the use to CAR T cells in central nervous system tumors. Dr Hwang referenced a 2013 study by Hegde et al[24] that combined different CAR T cells types together to show increased response in combating tumors. Another study by Brown et al showed initial regression of adult glioblastoma after CAR T cell therapy; however, there was massive recurrence. Additional mouse models have shown that combining CAR T cell with BITEs will decrease glioblastoma size.

Tumor vaccines are another promising immunotherapy treatment modality; however, it relies heavily on the patient's immune system to stimulate an appropriate response. Keskin et al[25] in 2019 studied the efficacy of tumor vaccines. Tumor cells were retrieved surgically and then underwent genome sequencing and HLA typing to predict personalized HLA-binding peptides for each patient. The peptides were synthesized into long peptides pools that would be the most antigenic and then injected into the patient, hoping to provoke an endogenous immune response. These vaccines are administered with a costimulatory agent.

It is difficult for our bodies to recognize tumors for a variety of reasons, but one way to counteract that is to infect them with a virus the immune system can recognize in the form of an oncolytic virus. This may be quite effective as the human immune system is wired to innately and adaptively attack viruses. The other unique component to using viruses is that many of them already cause apoptosis; therefore, the infected tumor cells will die and then theoretically release viral particles that would spread and infect surrounding tumor cells, leading to continual immune system attack. There are many viruses currently under investigation for this therapy technique.

There are currently multiple immunotherapy clinical trials investigating the use of immune checkpoint blockade. These components may work singularly or in combination. The immune checkpoint inhibitors (ICIs) function to block antigenic tumors and reactivate the immune system to attack tumor cells. Bouffet et al[26] examined the effects of ICIs for hypermutant glioblastoma multiform from mismatch repair deficiency and found complete response when ICIs were used as a single agent; however, the durability of the response was unknown. This discovery could be promising for pediatric cancers that also have mismatch repair deficiency.

Immunotherapeutic trials have difficulty translating into clinical trials for multiple reasons. First, preclinical data with mice modeling have difficulty assessing immunotherapy and do not represent the clinical heterogeneity seen with human subjects. Second, many patients are on steroids or previously had chemotherapy, and are already immunosuppressed. In the previously mentioned study by Keskin et al in 2019 regarding tumor vaccines, they also assessed vaccine response in correlation with dexamethasone use. Patients that were currently using dexamethasone at the time of the vaccine had a poor immunologic response, whereas patients without dexamethasone use had a better response. Third, tumor pseudo-progression occurs with use of immunotherapy agents and is often a retrospective diagnosis. Wolchok et al[27] in 2009 studied melanoma response with ICIs that showed an overall 100% or better response with therapy, but realistically, patients had variable responses. Some patients had excellent overall tumor shrinkage, whereas others had some tumor growth and then tumor shrinkage. There was also a subset who had a 150% increase in tumor size and then primary lesion shrinkage, which could be problematic and indicate possible new metastatic disease despite decreased primary tumor size. This can be misguided and misleading when considering ICI and immunotherapy. There is difficulty distinguishing between pseudo-progression and real tumor progression because clinical symptoms will be the same and tissue analysis may show infiltration from the immune system from the immunotherapy.

The iRANO criteria was developed by Okada et al[28] in 2015 to help guide clinicians through clinical trials with immunotherapies. The criteria established continuing immunotherapy trials if patients are clinically stable, despite imaging or symptoms that might indicate progressive disease, as those might be suggestive of immune cell infiltration versus actual tumor progression.

In conclusion, immunotherapy is revolutionizing the pediatric cancer landscape; however, responses in pediatric central nervous system malignancies have not yet been consistently achieved. There are no good predictive markers for patients and there are challenges to immunotherapy clinical trial design.

Late Effects: Prevention and Management

Elizabeth Wells, MD, MHS; Brain Tumor Institute, Children's National Medical Center, Washington, DC. Dr Elizabeth Wells presented the prevention and management of late effects in pediatric brain tumors. Seventy-five percent of pediatric brain tumor patients are now long-term survivors, and many suffer from long-term medical, psychosocial, and neurocognitive problems that impact their overall quality of life. Late-term effects occur 5 years from diagnosis and are dependent on multiple factors, such as tumor type and location, age at treatment, chemotherapy/radiation regimen and intensity, and tumor progression and recurrence. It is theorized that the mechanism is a result of direct injury and cell loss, as well as response to that injury through oxidative stress and tissue inflammation. These effects are vast and may affect multiple body systems, leading to neurologic, endocrinological, and psychiatric issues.

Dr Wells highlighted a study of hers, in conjunction with Roger Packer, that longitudinally assessed the late-onset neurologic conditions in survivors of pediatric brain tumors. They assessed 1876 survivors who were diagnosed between 1970-1986 and followed them for 30 years. The study concluded that late neurologic effects cumulatively increased over time and survivors had a greater risk of adverse neurologic consequences in comparison to their siblings.

It is best to follow pediatric brain tumor patients in multidisciplinary clinics, so that practitioners know how to better approach these survivors and help them appropriately. These clinics provide symptomatic management and information about survivor resources to patients and their families. Unfortunately, only 11% of pediatric brain tumor survivors are followed in multi-disciplinary long-term follow up clinics.

The COG long-term follow-up exposure-based screening guidelines are evidence-based guidelines that record potential late effects, how to evaluate them, and what interventions should be considered. The psychosocial and quality of life section documents that cancer exposure is a risk factor for mental health disorders and suicidal ideation. Brain tumor survivors often struggle with friendships, relationships, and integration into their community more often compared to other cancer survivors and their siblings. Cranial radiation exposure is another guideline considered and evaluates the potential for secondary malignancies. It recommends annual neurologic examinations for surveillance and the use of MRI when clinically indicated. There are multiple studies in the current literature to suggest stroke screening of pediatric brain tumor survivors. It is mostly a small vessel cerebral vasculopathy, but a 2018 study by Nordstrom et al[29] showed that 10 of 115 survivors of craniospinal radiation therapy had asymptomatic large vessel cerebral vasculopathy approximately 6 years post-treatment. Interventions may include increasing health screening for comorbid factors like hypertension, dyslipidemia, and diabetes. Strokelike migraine after radiation therapy syndrome (SMART) often occurs in the distribution of the radiation field and presents with strokelike symptoms. MRI shows focal cortical T2 hyperintensity and gyro enhancement that is similar to posterior reversible encephalopathy syndrome (PRES).

The neurocognitive deficits associated with pediatric brain tumor survivors is quite vast. The COG guidelines suggest screening for educational and vocational progress with referral to neuropsychology at the beginning of long-term follow-up clinic and as needed. Current interventions include educational support, pharmacotherapy, cognitive remediation, and exercise. The current standard-of-care educational support is a 504 plan or individualized education program (IEP), where accommodations may include extra time for assignments/exams, quiet setting, reduced workload, and using assistive technologic devices. The effectiveness of these measures has not been studied. Educational support is usually instituted once a patient shows poor academic progress and requires medical professionals to advocate if it is needed earlier. The best studied pharmacotherapy for cancer treatment–related neurocognitive impairment are stimulants, specifically methylphenidate. As in children with attention-deficit hyperactivity disorder (ADHD), it improves attention, processing speed, and short-term memory; however, it has questionable benefits for academic performance. An agent currently being considered is memantine. It is an open NMDA receptor blocker that functions to restore long-term potentiation and improves learning in excitotoxic states. This drug is already used to improve cognitive function in adult patients with vascular dementia and Alzheimer's dementia. There has been some evidence of neuroprotection from cranial radiation therapy in adults with brain metastases. The last drug being considered to aid neuroprotection is metformin. Metformin activates pathways to enhance neural precursor cell proliferation and differentiation. Preclinical studies show a protective measure against focal cerebral ischemia by improving demyelination and hippocampal neurogenesis.

Cognitive remediation has shown significant progress in treating neurocognitive effects and recently moving toward prevention strategies. A 2008 study by Butler et al[30] conducted a randomized clinical trial of a problem-solving intervention where pediatric cancer survivors attended sessions with a therapist at the hospital 2 hours per week for 6 months. Sixty percent of the patients completed this program and the results showed modest gain in achievement, attention, and memory. It showed significant improvement in writing samples and social skills. Cogmed is a home-based, computerized working memory training program that was used as an intervention in a randomized clinical trial by Conklin et al[31] in 2017. Patients completed 25 sessions over 5-8 weeks that were 15-45 minutes in length. Participants saw improved visual and verbal short-term and working memory that lasted approximately 6 months. Physical exercise has also shown to improve cognitive impairment in pediatric brain cancer survivors. Riggs et al[32] in 2016 studied 28 children (aged 8-16) with brain tumors treated with cranial radiation therapy. Each child was prescribed an exercise program consisting of 1 hour of aerobic exercise 3 times per week, lasting 12 weeks. Participants had an increase in physical fitness between 11% and 49%. Additionally, there was increase

in white matter fractional anisotropy. Sport-related complications are rare in children with central nervous system tumors, and the benefits outweigh the risks. Recommendations vary and should consider individual risk factors, such as having a ventriculoperitoneal shunt.

Pediatric brain tumor therapy has changed to improve survival and reduce toxicity. This is noted with improvement to surgical techniques, delaying cranial radiation, reducing radiation dose and field size, and the introduction of proton beam and biologic therapies. A 2016 study by Lafay-Cousin[33] et al showed that delaying or avoiding radiation therapy in young children with standard-risk medulloblastoma and treating them with high-dose chemotherapy spared neurocognitive effects, and still resulted in good survival outcomes. Studies with proton beam radiation have shown less IQ decline in comparison to photon beam therapy, but this continues to be studied closely. There is little evidence about biologic agent's ability to prevent neurotoxicity as they are newer and currently being studied.

A major complication of postoperative treatment in medulloblastoma is cerebellar mutism syndrome or posterior fossa syndrome. This is characterized by postoperative mutism, hypotonia, ataxia, and severe emotional lability. Patients have worse long-term cognitive outcomes despite supportive care and rehabilitation. Current studies assessed whether changes to surgical techniques may result in better postoperative outcomes. At her home institution, Dr Wells explained, they reduced the amount of cases with cerebellar mutism syndrome by altering surgical techniques to involve less retraction via a cavitational ultrasonic surgical aspirator (CUSA), and using a telovelar approach versus the standard transvermian surgical approach.

There is a paradigm shift in assessing and preventing neurotoxicity in pediatric brain tumor patients. Traditionally, screening and evaluations were conducted after therapy and tended to focus on post-treatment interventions with remediation, but now, universal monitoring has begun at the start of diagnosis. These patients experience life-altering obstacles, and healthy lifestyle screening and symptom management may prevent and reduce late effects. There have been changes to primary prevention and treatment to prevent neurotoxicity, as well as introduction of neuroprotective agents to study pharmacologic prophylaxis. Continued research, education, and community efforts will help improve the quality of life for these survivors.

Session IV: Future Directions Panel Discussion and Question and Answer Session

Audience: I have been working in the field for a long time and thank you very much to Dr Maria and all the participants. I was very enlightened and I'm very grateful we are having this discussion here at NDC and at the Child Neurology Society Meeting. What is the role of the child neurologist in this field in the past, current, and future? How do we stimulate our trainees to enter this field? Are neuro-oncology fellowship programs training child neurologists as much as pediatric oncologists to enter this field?

Dr Packer: In our fellowship program, 50% of our trainees are child neurologists and 50% are pediatric oncologists but I think it is hard for the child neurologist to not become a fifth wheel during the process; they have to be very involved in the therapeutic decisions. There's nothing specific that says a child neurologist can't give an MEK inhibitor, and by the way, they will in treating children with neurofibromatosis who in practice will not all go to the oncologist. So, there are a lot of ways they can be involved in developing therapies and studying basic mechanisms of disease.

Dr Wells: I would like to add to that. So at diagnosis, patients are often treated on the neurology service, in the ICU, and we are integrally involved in developing protocols and in management.

Audience: Let me just respond very briefly to these thoughts; we do not have a curriculum for trainees and they do in the adult neuro-oncology and actually have credentialing by the United Council of Neurologic Subspecialties. And in the sense, we have been somewhat negligent in credentialing graduates of our fellowship programs.

Dr Hwang: We had a meeting with all the child neuro-oncology directors about 5 years ago now, and we thought about what we would need to move forward with neurology trained neuro-oncology providers, not only at our institution but at every institution. What came about consensus wise, although there's an effort now to revisit that issue, is first, we thought UCNS certification was difficult for a pediatric facility to get from a neuro-oncology standpoint because one of the criteria to sit for the boards is to be trained in a UCNS certified program with adult neurology and adult neuro-oncology program.

Audience: I think there is a difference between neurologists trained as neuro-oncologists and then making sure our child neurology trainees are trained effectively to recognize acute and long-term consequences of treatment for childhood brain tumor or childhood cancers. And this is particularly important because in this day and age, 1 in 250 adults are survivors of a childhood cancer, so because of our effective therapies, those individuals are living longer and longer and longer; and therefore, the cumulative toxicity that we're seeing, in not only brain tumor survivors, but also childhood cancer survivorship is really increasing over decades. Dr Wells showed the results of the CCSS study, so it's really important that child neurology residents are trained not only to recognize issues at an inpatient setting but also at the outpatient setting.

Dr Jabado: I believe that pediatric oncology should really recognize the potential long-term complications of survivorship.

Dr Maria: As neurologists, our first involvement clinically is when a patient has presented to the emergency room with a tumor. Second, we can be involved postoperatively and posterior fossa syndrome can be seen. I have a question about this syndrome. I thought that some of the modeling on posterior fossa syndrome that was showing disruption of ascending information via superior cerebellar peduncles from the cerebellum is to blame. And I wondered whether some of the elegant imaging we saw earlier this morning could help study the issue and identify those at risk of the complication?

Dr Wells: Yes indeed. DTI studies showing postoperative changes to the cerebellar-dento-thalamo-cortical tracts. There is no intraoperative monitoring yet that might help, but intraoperative MRI may provide information on the tracts.

Dr Packer: There are some tumors where the risk of mutism is higher. It is also true that the rate of mutism varies by institution and surgeon.

Dr Wechsler-Reya: I actually just want to add to that as one of our K-12 scholars, from Iowa, just published a paper as a last author 1 month ago in *Neurology* explicitly looking at the incidence of posterior fossa syndrome or CCAS, and finding even though we think the rate's being 10% to 20%, it's actually directly related to how much the outflow tract gets interfered with a large lesion base. So, at the higher end, if you hit 100% of the outflow tract, your rates expanded to 50% and that the lesions that avoided the tracts actually had rates near zero. So, there is a direct correlation between how much outflow tracts you're hitting with the resection. These are based on quantic scan tracings, so it's very frightening.

Dr Wells: That's very interesting. I wish there were a way during surgery to guide that.

Audience: I had a provocative question for . . . do I ask it or not ask it? (*crowd laughter*) . . . Are mouse models necessary?

Dr Wechsler-Reya: Most immunotherapies have not been tested in preclinical models, because there have not been preclinical trials to test them. Most of the drugs that historically have gone into clinical trials have not been tested extensively in models, and certainly not in the kinds of models we're talking about. I think there's going to be cases where a drug is ready to go into trial and people will push it in without having preclinical data. I think the danger in doing that is that we run a lot of trials, and of those trials, many of them will fail, and we won't really know why. And I'm not saying we will know why because of doing preclinical studies, but in some cases we will. As Roger said, and I think this is equally or more important, I think that we need to design our trials so that we learn more from them even if when they fail.

Audience: How accessible is proton beam therapy? How will radiation change with molecular data?

Dr Merchant: Progress we have made in pediatric radiation oncology has been truly incremental, not major changes over time. If I go back to all the years that I've practiced and I'd tell you we've made a 5% change at every year by incorporating multilead columnation, by improved targeting, by reducing the target blind margin, by changing this or that, I mean, 5% per year adds up to something fairly substantial over time. The challenge for us is not for us to design a trial that matches molecular data therapy, it's we have to understand what happens to the patient in terms of the molecular subgrouping. What is the pattern of failure? This is really important to the radiation oncologist. And when we see in a study reporting event-free and regression-free survival, that tells us very little; did our treatment fail because we didn't treat the neuro-axis—we only treated the primary tumor, or did we not treat the primary tumor with a sufficient margin in the dose? The pattern of failure for us means so much. So I think for the reporting on some of the major trials and even single-institution trials, we then have to go back and match the radiation dose with failure and the change. You have to look at molecular subgrouping and you can't forget the basic clinical pathologic information. It is not going to be just the tumor that drives the treatment, but it's also going to be the host. What are the predisposing factors to radiation effects? And I think we're sort of on the cusp of that because we've collected, not just for tumor information, but also information about the normal tissues. So, I think it all comes together and what we do is incremental, my wish would be that we enroll more patients on clinical trials.

Dr Maria: My question is about pseudo-progression with proton beam therapy. Is this something that you've seen more of with proton beam relative to nonproton? And, I thought protons were to provide a very restricted field of therapy, but we're seeing pseudo-progression in a field of radiation that's bigger than I thought it would be for a lower-grade tumor that's progressing.

Dr Tom Merchant: Proton therapy has a radiobiologic effect that's greater than photon beams. And what we do or what we've done is we've set the differences at 10% and so we've scaled back the physical dose, so that the 2 beams match each other in terms of biologic effect. So that's just an estimation, it's not entirely accurate. There's potential that it's 20% to 30% difference under certain conditions, and those conditions include the physics of the treatment, how deep the tumor is, how big the tumor is, what the energy of the beams are, but it could also depend on the patient because every patient is different. So, the responses to normal tissues in tumors are probably different and there's an underlying biology that I think we're also . . . I think that also imaging has gotten so much better over time, more sensitive to some of the changes that we see. So I think there's sort of a combination of things there—there's the environment, the way you pay attention to things. I think the tumors . . . there's always been pseudo-progression, um, but if you have a beam that is strong than the beam we used to use, I would expect such things to happen not just in the tumor, but also in the normal tissues. I'm sure Roger could speak to that.

Dr Packer: We've gone back and forth on this, and I do think that those physicians who are taking care of children who are being referred especially to new proton beam therapy I'd be very aware that there have been reports, including ours, some public, some not, of significant damage after proton beam radiation. I think it's complex. Often those children use quite a bit of chemotherapy. There's been a lot of issues, but it's not that easy and we do think it's a useful technique because it limits the volume affected by radiation, but there are risks there and I'm always concerned about the very young child and the radiation they receive, but there's times they do very well so I think it can be difficult.

Dr Merchant: I just want to emphasize one other point that was mentioned a few minutes ago about capturing imaging before and after surgery to identify patients who might be at risk for side effects. I don't think we do enough to incorporate sophisticated imaging in our treatment plans to identify tissues that appear to be injured. And could we do a better job at avoiding tissues that are injured and reduce side effects. I think there's so many areas or opportunities for improvement there.

Dr Packer: Just one other thing. . . . If you look at the CAR-T data that's coming out, when they looked retrospectively when a

patient had some evidence of injury before the CAR-T therapy to the brain, those patients sometimes developed irreversible damage. Sometimes we don't get much choice, even if there's brain stem injury that can be avoided by therapeutically treating the child.

Audience: I have a question that relates to the earlier topic of clinical trial enrollment. So say I'm a parent with child who has a brain tumor, and there's 20 different trials I could enroll my child into. What are the ethical implications of having all these options that are not right for the patient, you know if they're not all equivalent? How do we in the field, guide parents make these decisions properly?

Dr Packer: I think that's a great question. I would love to hope we'd have 20 options for every child, and you are absolutely right. In DIPG there's often 2 or 3 options. I do believe you have to be honest with yourself if you have a phase 2 study. In theory if that study has past toxicity, then it should probably be prioritized before phase 1 to treat the child. The second is that I think it's very important for an institution—and we don't do a good job of it—we used to do a better job to have better internal prioritization of "this is our first option, but we have to tell you what the other options are, and this is why this is our first option." I do think it's incumbent on the physician to not give people 3 options and say "you choose"—that's probably the worst role of a physician in this process. You have to make a recommendation and why you make the recommendation, and hopefully it's an unbiased one based on some data. So that's my bias. I still think the reason we saw a drop in clinical trials because there's a lack of interesting studies. Interesting studies are about to open, we'll hopefully see a boost up in clinical trials, and also because these trials last so long, and there's fatigue from the investigator after something's been open for 7 years, why should I enter another patient?

Dr Wechsler-Reya: I just want to point out, that in addition to whatever advice you give the patient, they're getting, as you may already know, social media advice, networking advice from lots of other people. So the challenge is not just what do you tell them, but how do you tell them how to handle all that information. I think, certainly the physicians I've talked to, that's one of the biggest challenges.

Audience: Would lorazepam be helpful in treating posterior fossa syndrome?

Dr Wells: Psychopharmacology has not been well studied in cerebellar mutism syndrome. Anecdotally, we see drugs like valproic acid and less from SSRIs [selective serotonin reuptake inhibitors] that should be better explored in the molecular pharmacology.

Audience: Can we hear more from Dr Hwang about immunotherapy?

Dr Hwang: The preclinical models of immunotherapy have not so far been a prerequisite and there are a lot of initiatives and trials right now going up-front with immunotherapy in particular. So DIPG is clearly a wonderful example of that where children would not make it past 1 year or 2 and so there are already consistent trials using TK1 inhibitors and radiation therapy. There's a platform from the University of Florida that is using inhibitor therapy plus vaccines plus adjuvants. Patients can have such bad prognosis that I think it's reasonable to give them a shot at something that might transform their outcome, because right now it's terrible. There will be other types of tumors, and as Dr Merchant was detailing about how we learned so much about medulloblastoma, and they're being put into several different group categories but there are several subgroups that essentially have neuroprotective survival genes. I think there will be other tumors that join the fray that will not only include immunotherapy but also targeted therapies and other elements like that.

Dr Schor: Yea I think from the standpoint of agents penetrating into not just the central nervous system but into the region of the tumor, and comparing penetration into the tumor with penetration into normal surrounding tissue. I mean, the technology of micro-dialysis catheters and things like that is increasingly good and less invasive, less injurious. There may also be imaging modalities that allows us to visualize agents getting into the tumor and even less invasively than that. The other thing that biopsy also potentially allows you to investigate, how hospitable the environment is to the growth of the tumor, and are there factors that you could detect that aren't in the tumor itself, but that are allowing the tumor to implant, and grow, and thrive. And I think the leukemia biologists have shown that macrophages and macrophage precursors sitting in the bone marrow feeding this tumor that usurps their machinery. I think the same thing is likely happening in the brain.

Dr Wechsler-Reya: I agree about the importance of biopsy and, in the context of immunotherapy, did something happen when the drug got in? If it's a small molecule and you're looking for the biomarker effect, that's great, but if you're looking for data checkpoints inhibitors, increasing activity of T cells, are there more T cells in there—I think the only way we'll be able to do it is by studying the immune-micro environment and studying it before and after the therapy.

Dr Packer: Before we jump to that conclusion, the drug that doesn't get into the brain has a mechanism. They are the drugs with the best efficacy to date of any drug we've trialed in the past 20 years. So, I think we really need to be careful of when we say "the drug doesn't get into the brain"—it's the percentage of the drug when it's on the target effectively and it very well maybe, and there's going to be data coming out relatively soon from a couple of laboratories, that the MEK inhibitors are better at lower concentrations than at high concentrations.

Dr Wechsler-Reya: I guess that point is that not some drugs can't get there and have some benefit, but the amount you have to use maybe—toxicity is going to be of some concern here. In adult tumors, for example, 10% to 20% cover the brain, that's quite a large population of patients, then the drug companies should have some incentive to go after it.

Dr Packer: I just think we just need to be smart about how we even interpret drug concentrations, and I agree completely about how it isn't drug concentration alone, it's also the biologic effects.

Audience: I wanted to go back to the cerebellar mutism syndrome question. There's a beautiful article that was recently published talking about the incidence of mutism in different medulloblastoma subtypes and the reason for, as we predicted,

because of their invasiveness, were the ones who had the most toxicity. So not only did they have the worse survival, but they also have toxicity. We've been using zolpidem—we've published one case report that really was astounding to us.

Dr Wells: I think that's great, and there's, um, an international posterior fossa society, so there's a nice network to share these stories and to explore further. And again, a role of the child neurologist in terms of early rehab, early mobilization, and having discussions with the family about the role that enteral or tubal delivery of medications is truly critical to explore.

Dr Maria: I have 2 questions, one that has to do with what we covered earlier in the day on methylation map and the whole frameshift pathologically that we're dealing with, and the second has to do with the immunotherapy. So, with the first one, Rob—the way, I guess I understood it, and correct me if I'm wrong, is that the methylation status gives us a snapshot of where that child's cells basically stalled in development. And that snapshot, even in the future, when the tumor is resected, reflects on the time of origin of the tumor. Is that correct?

Dr Packer: I think that's the theory and I think there's a lot of reasons to believe that is the case. I'm speaking at the risk of overstepping my knowledge relative to Paul and David, but I think there's been enough analysis of methylation during development and during differentiation along cell lineage to know that the methylation profile of a cell changes as it goes from a multipurpose cell to lineage-restricted progenitor to a differentiated cell. And now when you look at the profiles of tumors and you see similarities, it's provocative to think this methylation profile is a characteristic of the cell at the time it was transformed. It may be more complicated than that. I guess the question is "what's the purpose for which you're using methylation analysis?" I mean, one way to think about this is it's a fingerprint characteristic of the tumor across now, what was it, 50 000 samples. Whatever the reason is that is a stable characteristic or a common characteristic of group 3 medulloblastoma or posterior fossa ependymoma, it's a useful characteristic. I remember as a psych undergraduate, we learned about the Minnesota Multi-Phasic Personality Inventory, which was a whole series of questions that were administered that correlated with a patient's clinical profile, and some of those questions had nothing to do with the patient's actual profile. Like rutabaga was associated with a patient's psychiatric profile. And so, at the very least, I would say this is something like that. There's a very strong correlation between the biology and the phenotype of that tumor, but I do think that as we acquire more of these data and we look at them not just as diagnostic tools, but actually as reflective of what's going on in a tumor, we're going to learn a lot more. And so, I hope that those kinds of studies, where you look not just at the fingerprint and say "this looks like a PFA," but actually look at what is this telling us about the pathways that are activated or inactivated. That's going to be really important.

Dr Maria: Just as a follow-up . . . It's really the case that we have, at least for now 82 types based on the methylation, but I don't understand is why, when methylation is a measure of gene expression in either situation, why the methylation status isn't reflective of function? Why is it that genes that are either hypo- or hyper-methylated that are seen on that fingerprint? Why is it that we don't know or don't think that they have activity in the way of pathogenesis?

Dr Wechsler-Reya: I don't think that it's not the case that this correlates with gene expression—I think it does. I think the correlation is simply not as simple as hyper-methylated regions or hypo-methylated regions are all active. I think there are other things going on, and so, looking at these data now more carefully, I think it's going to be really important to draw those lines and figure out which part of the genome methylation signatures is actually telling us something of function.

Dr Jones: I basically agree with that. I think you already answered the question quite well earlier. The methylation itself is already one small aspect of the whole mechanism, which is controlling gene expression. We have all the histone and chromatin marks, as well as different levels of activity and transcription factors, so even if we have regions which correlate early that's only a fraction of the actual gene expression. The part of it is also the design of the assay itself. Fortunately, most of the probes were designed to fall into the promoters, and it now turns out those regions were not the regions which correlated most closely with gene expression. So, if you wanted to do a study where you were trying to correlate with methylation and gene expression, then microarrays are fortunately not the best method to be doing that.

Dr Maria: Then my other question has to do with T cells. I'm trying to reconcile part of what Dr Gutmann said, which was that T cells were an integral part of tumor progression within the optic pathway tumors. But I also heard that medulloblastomas depleted the T cells. So, I was trying to reconcile those 2 pieces, like we're talking about immunotherapy, but are T cells playing a role in other tumors besides the optic pathway?

Dr Wechsler-Reya: So, I don't know where David (Gutmann) is, but there are 2 things I want to emphasize from my point of view. One, every tumor is different, so T cells may be playing pro-tumorigenic role in optic pathway glioma, and it may be playing an anti-tumorigenic role in other settings. Second, the notion that medulloblastomas are devoid of T cells—I think is based on potentially a misperception on how many T cells you need to get an immune response started. I think that to get a T cell response, you need antigens and those antigens to be presented, meaning T cells need to be somewhere in the neighborhood, and you need them to be activated. But this is a positive feedback loop, once it gets started, which is why I think you get cytokine release syndrome. You get lots of T cells doing what they wouldn't otherwise be doing. So, I don't think we should be quite referring to tumors as "hot or cold," I think we need a more sophisticated understanding of what we need, in terms of each of the parameters we need to get in immune response. I think Dr Hwang did a fantastic job of defining the 4 or 5 or 6 things you need—broad categories, and things you need—and I think from the standpoint of making immunotherapy effective, we're going to need to look at each patient or at least each subtype of tumor, and say "what's missing here? What do we need to get to the point where the immune response will take place?" I don't think you need a lot of T cells, you just need some.

Dr Wells: A question that's coming up in terms of T-cell toxicity and cytokine release syndrome is the utility of cerebrospinal fluid analysis and cytokine analysis, and I think that's

especially important. We're doing lumbar punctures in these patients due to high pressure. There's a lot of opportunity to get a lot of fluid available to check for cytokines, but it's not really clear if that would be useful. So what are you thoughts on banking or assessing cytokines when we're already doing lumbar punctures for clinical purposes?

Dr Wechsler-Reya: I think it's a great idea to bank them. CART T cells might produce a cytokine release that gets into the cerebrospinal fluid, but dendritic cells activation might not, and so, I think we should be banking, at the very least be banking those or looking at them to see if they're useful correlates.

Dr Maria: Before we wrap for the day, one of the things we try to do in NDC every year is to try to identify some key unanswered questions. We have a lot of young investigators here, and it would be good for them to hear that from you—and from many of the other speakers who may not be up front. What do you think, Tom (Merchant), in your field, with regards to what we covered today, is a key unanswered question from a radiation perspective?

Dr Merchant: I think we're well into the use of proton beam therapy and we still can't answer the question of whether or not it's better than photon therapy. At least we know there's induction and acute effects—if you're not, then you're irradiating the chest, abdomen, and pelvis. When you're getting to spinal radiation, does the use of anti-emetics go down and the child tolerates treatment better and recovers from surgery and so forth? And I think we've seen that. We haven't really quantified that as well as we should, and that's because we didn't collect that data as well when we did the photon therapy. The more common side effect is the neurologic and endocrine, cognitive effects—we don't have any evidence of that yet. So, I'm a bit concerned, but if we're driving the target volumes smaller and smaller, and we're reducing dose even with enhanced photons techniques, it's going to be difficult to show the difference. But maybe in the 10, 15, 20 years down the road, when we've irradiated less normal tissue, some of the rare, and really devastating, complications we see in long-term survivors, and this is pointed out in earlier presentations, maybe that will be the key. We need to enroll children in clinical trials, especially those clinical trials with toxicity assessments.

Dr Wechsler-Reya: I guess I'd like to harp back to the question Nada (Jabado) asked about do we need clinical data to move forward in a clinical trial, and I'd like to get to the point where we're not asking that question. And where the investigators who are capable of doing preclinical trials are working closely with the investigators who are designing clinical trials to make sure that those efforts are harmonized. That we are testing the agents that are the highest priority, that no clinical trial gets written and launched overnight. I'd argue that there should never be a situation where we can't test something preclinically before going into a clinical trial. So, I guess my suggestion, if not question, is that more preclinical investigators—people who are doing more preclinical work—work more closely with people that do clinical trials, so those efforts are harmonized and synergized with one another, rather than working across purposes.

Dr Schor: I think one of the things that came across loud and clear throughout the entire day, to me, is the extraordinarily robust level of complexity of the tumor system that we're dealing with. But, if you think about this as a development operation, and a developmental arrest, rather than as a de novo, dedifferentiation if you will, of a previously normal cell, it shouldn't be all that surprising. I think if I had one thing to do or to answer—many of you know that I'm at the NINDS, and we're leading the brain initiative with our colleagues at NIMH—and one of the things the brain initiative is currently trying to do is to create a spatial cell map to actually map out both from self-phenotype and from cell transcription, if you will, to map out what kinds of how many totally different kinds of cells are there, and in which places in the central nervous system. But one thing we are not currently doing—that I've been pushing for—is what happens to that map during development?

Dr Packer: As regards to the molecular targeted therapies, and we keep coming back. We have to be able to design trials and figure out why we failed—we have not done that. We must design trials to try to figure out where we put these agents, and to prove that they're effective. And we have to design follow-up trials to figure out the long-term effects of these molecular targeted therapies on brain development and other organs. We need to do them now or we won't have the data when we need to know how to incorporate them to replace therapies. The other thing we need to know how are we going to get these new drugs to the brain? What is going to be the role of things like low-intensity/frequency ultrasound to open up the blood-brain barrier? Is there any role for conventional delivery this early on or are we just wasting our time? Do we need to only look at agents we need to get into the central nervous system in our intact models? Or are there other ways that we haven't figured out?

Dr Wells: I think a key question would be whether neuroprotective agents or techniques can be introduce earlier in the course of treatment to reduce side effects.

Dr Maria: Any other thoughts?

Dr Hwang: I heard the comment about young investigators having very tangible initiatives and I think that one of the dangers when there's so much that's unknown is that it's so complex and hard to break up in bite-sized elements. There's one thing, just in this conversation that I heard that was interesting, is that Beth (Wells) asked Rob (Wechsler-Reya) "What do I do with this CSF that we're drawing from all these kids?" That's not really an interventional therapeutic option opportunity, although it could be an opportunity for diagnostics like self-DNA. It could also be a bite-sized opportunity, and I was just in the PICU with an oncology patient who was status-post CART T cells and profoundly encephalopathic, and I had no idea what to do—Do I give them an MK inhibitor? So doing a study just to see who gets better with what and intervening with particular cytokine interventions can be really interesting from a neurology standpoint.

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